ZooKeys 1187: 1–29 (2023)
DOI: 10.3897/zookeys.1187.104536

Research Article

A new cryptic species of terrestrial breeding frog of the *Pristimantis danae* Group (Anura, Strabomantidae) from montane forests in Ayacucho, Peru

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Abstract

Based on morphological and molecular characters, we describe a new species of terrestrial breeding frog of the *Pristimantis danae* Group from montane forests of La Mar Province, Ayacucho Department in southern Peru, at elevations from 1200 to 2000 m a.s.l. The phylogenetic analysis, based on concatenated sequences of gene fragments of 16S rRNA, RAG1, COI and TYR suggests that the new species is a sister taxon of a clade that includes one undescribed species of *Pristimantis* from Cusco, *Pristimantis pharangobates* and *Pristimantis rhabdolaemus*. The new species is most similar to *P. rhabdolaemus*, which differs by lacking scapular tubercules and by its smaller size (17.0–18.6 mm in males [n = 5], 20.8–25.2 mm in females [n = 5] in the new species vs. 22.8–26.3 mm in males [n = 19], 26.0–31.9 mm in females [n = 30] of *P. rhabdolaemus*). Additionally, we report the prevalence of *Batrachochytrium dendrobatidis* (Bd) in this species.

Resumen

Describimos una nueva especie de rana terrestre de desarrollo directo del grupo *Pristimantis danae* de bosques montanos procedentes de la provincia de La Mar, departamento de Ayacucho al sur de Perú con rango de distribución altitudinal entre los 1200–2000 msnm, en base a caracteres morfológicos y moleculares. El análisis filogenético basado en las secuencias concatenadas de los fragmentos de genes ARNr 16S, COI, RAG1 y TYR sugiere que la nueva especie es un taxón hermano del clado que incluye a una especie de *Pristimantis* no descrita de Cusco, *Pristimantis pharangobates* y *Pristimantis rhabdolaemus*. La nueva especie se asemeja más a *P. rhabdolaemus*; de la cual difiere por la ausencia de tubérculos escapulares y su menor tamaño corporal (17.0–18.6 mm en machos [n=5], 20.8–25.2 mm en hembras [n=5] en la nueva especie vs 22.8–26.3 mm en machos [n=19], 26.0–31.9 mm en hembras [n=30] de *P. rhabdolaemus*). Adicionalmente, reportamos la prevalencia de *Batrachochytrium dendrobatidis* (Bd) en esta especie de Terrarana.

Key words: Chytridiomycosis, cryptic species, montane forests, morphology, phylogeny

Palabras clave: Bosques montanos, especies crípticas, filogenia, morfología, quitridiomicosis

OPEN ACCESS

Academic editor: Angelica Crottini
Received: 4 April 2023
Accepted: 21 August 2023
Published: 20 December 2023

ZooBank: https://zoobank.org/226A24AB-B4BE-4EFD-BF11-D6CA719AB601

Citation: Herrera-Alva V, Catenazzi A, Aguilar-Puntriano C (2023) A new cryptic species of terrestrial breeding frog of the *Pristimantis danae* Group (Anura, Strabomantidae) from montane forests in Ayacucho, Peru. ZooKeys 1187: 1–29. https://doi.org/10.3897/zookeys.1187.104536

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Introduction

Pristimantis (Terrarana, Strabomantidae) is an amphibian genus that comprises more than 600 species and occurs thoughout the Americas (Hedges et al. 2008; Padial and De La Riva 2009; Padial et al. 2014; Waddell et al. 2018) from Honduras to Argentina. In Peru, there are 145 *Pristimantis*, which represents more than 20% of its global richness (Frost 2023). Many species of *Pristimantis* are morphologically similar despite belonging to different lineages (Elmer and Cannatella 2008; Padial and De la Riva 2009; Siqueira et al. 2009; Kieswetter and Schneider 2013; Hutter and Guayasamin 2015; Ortega-Andrade et al. 2015). The ubiquity of cryptic species in *Pristimantis* has led to underestimation of the real number of species in the genus (Ortega-Andrade et al. 2015; Guayasamin et al. 2017; Paez and Ron 2019; Carrion-Olmedo and Ron 2021). Nevertheless, the application of molecular techniques in an integrative framework (Dayrat 2005) generated a steady increase in species richness of *Pristimantis* (Köhler et al. 2022; Reyes-Puig and Mancero 2022). Integrative taxonomy uses more than one line of evidence and discipline to infer species limits (Simpson 1961; Wiley 1978; De Queiroz 2005; Schlick-Steiner et al. 2010; Aguilar et al. 2013; Hutter and Guayasamin 2015) and has become a critical tool to identify and delimit species as well as in understanding species formation (Aguilar et al. 2013; Minoli et al 2014; Rojas et al. 2018; Hillis 2019; Zozaya et al. 2019).

One group with cryptic species includes *Pristimantis rhabdolaemus*. The taxonomic history is complex because Lynch and McDiarmid (1987) synonymised *Pristimantis pharangobates* with *P. rhabdolaemus*, until Lehr (2007) again split these two cryptic species. Incorrect labelling of GenBank sequence EF493706 (uploaded during the period from 1987 to 2007 when synonymy was in place) of *P. pharangobates* as "*P. rhabdolaemus*" (Heinicke et al. 2007; Padial et al. 2014; Lehr et al. 2017a, b; Acevedo et al. 2020) contributed to taxonomic confusion. Furthermore, specimens from Bolivia incorrectly assigned to *P. rhabdolaemus* added more confusion. Despite this history, *P. rhabdolaemus* species limits have not been studied using integrative taxonomy.

Therefore, as part of a study of *Pristimantis rhabdolaemus* species boundaries, we collected direct development frogs from the montane forests of La Mar Province, Ayacucho Department. Molecular and morphological analyses revealed that the collected specimens belong to an undescribed species. Our goals are to describe the new species and infer its relationships with other species of the *Pristimantis danae* species Group, as well as provide information about infection by the fungus *Batrachochytrium dendrobatidis* (Bd).

Materials and methods

Fieldwork and voucher specimens

VHA conducted field research in the montane forest of two small valleys (Fig. 1) in the VRAEM (Spanish acronym for Valley of the Rivers Apurimac, Ene and Mantaro), Ayacucho Department, southern Peru. The fieldwork was organised in two stages. The first occurred from November 2018 to June 2019 in the valley of the Chunchubamba River, which goes from Chiquintirca (2900 m a.s.l.)

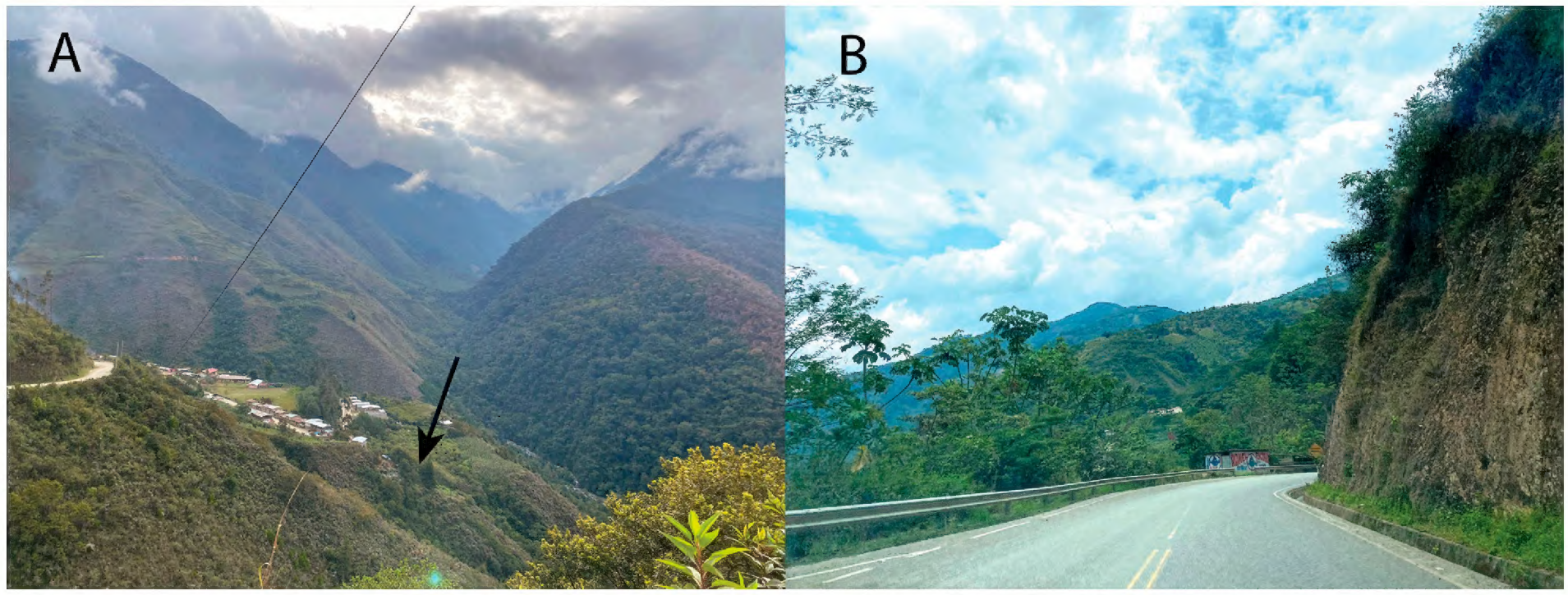

Figure 1. Montane forest of Cajadela (**A**) and Machente (**B**), Ayacucho Department. Type locality of *Pristimantis similaris* sp. nov. in Cajadela (**A**). Note the presence of secondary forest in both localities. Photo **A** taken on 8 November 2021 and **B**, on 24 October 2022. Arrow marks the type locality.

to San Antonio (800 m a.s.l.) in the districts of Anco and Anchihuay (both from La Mar Province). The second field-trip was in November 2021 in the valley of the Piene River, which goes from Yanamonte (2900 m a.s.l.) to San Francisco (800 m a.s.l.) in the Districts of Sivia (Huanta Province) and Ayna (La Mar Province), which included the visit to the type locality of *P. rhabdolaemus* in Machente (1650 m a.s.l), also previously known as Huanhuachayocc, a name no longer recognised by the locals.

We extracted liver tissues by pulling the liver out of a small abdominal incision. We fixed specimens in 10% formaldehyde and stored them in 70% ethanol in the Department of Herpetology of the Museo de Historia Natural Universidad Nacional Mayor de San Marcos (MUSM), Lima, Perú. The Ministry of Agriculture issued research, collecting and genetic resources permits (000063-2018-GRA/GG-GRDE-DRAA-DFFS-D, 029-2016-SERFOR-DGGSPFFS and D000012-2022-MIDAGRI-SERFOR-DGGSPFFS-DGSPFS).

Morphology and morphometry

We follow Lynch and Duellman (1997) for the description format, except that we use “vomerine odontophores” instead of “dentigerous processes of vomers” (Duellman et al. 2006) and Duellman and Lehr (2009) for diagnostic characters. We sexed specimens by examining for the presence of vocal slits in mature males and by inspecting gonads in dissected specimens. VHA measured the following distances to the nearest 0.1 mm with digital calipers under a stereomicroscope: 1) snout-vent length (**SVL**), 2) tibia length (**TL**, distance from the knee to the distal end of the tibia), 3) foot length (**FL**, distance from the proximal margin of inner metatarsal tubercle to tip of Toe IV), 4) head length (**HL**, from the angle of the jaw to tip of snout), 5) head width (**HW**, at the level of the angle of the jaw), 6) horizontal eye diameter (**ED**), 7) horizontal tympanum diameter (**TY**), 8) interorbital distance (**IOD**), 9) upper eyelid width (**EW**), 10) internarial distance (**IND**), 11) eye-nostril distance (**EN**, straight line distance between anterior corner of orbit and

posterior margin of external nares) and one extra measurement: 12) Finger IV disc width (**F4**). Fingers and toes are numbered pre-axially to postaxial from I–IV and I–V, respectively. We compared the lengths of toes III and V by adpressing both toes against Toe IV; lengths of fingers I and II were compared by adpressing the fingers against each other. Vladimir Díaz Vargas photographed live specimens and VHA preserved the specimens. We used photographs for descriptions of skin texture and colouration. Specimens examined are listed in Appendix 1; other collection acronyms are MUSM = Museo de Historia Natural San Marcos (Lima, Peru); KU = University of Kansas, Museum of Natural History (Kansas, USA); LSUMZ = Louisiana State University Museum of Zoology (Louisiana, USA).

Molecular phylogenetic analysis

We performed a phylogenetic analysis to infer relationships of the new species with other species of the *Pristimantis danae* Group (Padial et al. 2014). We used fragments of 16S rRNA, COI, RAG1 and TYR genes. We obtained novel sequences by extracting DNA from six specimens of the new species (MUSM 41030, 41031, 41035, 41036, 41037, 41323) with a commercial extraction kit (IBI Scientific, Iowa, USA). We followed Hedges et al. (2008) and von May et al. (2017) for primers and PCR thermocycling conditions. Primers are listed in Table 1. We purified PCR products using Exosap-IT Express (Affymetrix, Santa Clara, CA, USA). MCLAB (San Francisco, CA) performed Sanger sequencing.

We follow Padial et al. (2014) and Pyron and Wiens (2011) for species group assignment within *Pristimantis* and the choice of outgroup taxa. We downloaded from GenBank all available sequences of 16S rRNA, COI, RAG1 and TYR of other species of the *P. danae* Group and some of the outgroup taxa. We used selected species of the *P. conspicillatus* Group (*P. bipunctatus*, *P. iiap* and *P. skydmainos*) and *P. prolatus* as outgroup taxa (Appendix 2).

We used Geneious Prime version 2023.0.1 (Biomatters, http://www.geneious.com/) to assemble pair-end reads, generate a consensus sequence for each gene and align our novel and GenBank sequences with MAFFT included in Geneious as a plug-in. Posteriorly, we concatenated the four genes (16S, COI, RAG1 and TYR) using the default settings in Geneious. We trimmed aligned

Table 1. Primers employed in this study for PCR and DNA sequencing. F = forward, R = reverse.

Locus	Primer		Sequence (5' – 3')	Reference
16S	16SAR	F	CGCCTGTTTATCAAAAACAT	Meyer (2003)
	16SBR	R	CCGGTCTGAACTCAGATCACGT	
COI	dgLCO1490	F	GGTCAACAAATCATAAAGAYATYGG	Bossuyt and Milinkovitch (2000)
	dgHCO2198	R	TAAACTTCAGGGTGACCAAARAAYCA	
RAG1	R182	F	GCCATAACTGCTGGAGCATYAT	Heinicke et al. (2007)
	R270	R	AGYAGATGTTGCCTGGGTCTTC	
TYR	Tyr1C	F	GGCAGAGGAWCRTGCCAAGATGT	Lanfear et al. (2012)
	Tyr1G	R	TGCTGGGCRTCTCTCCARTCCCA	

sequences to a length of 591 bp for 16S, 685 bp for COI, 624 bp for RAG1 and 545 bp for TYR (Fasta file included in doi: 10.5281/zenodo.8278333). To obtain the nucleotide substitution model for each gene, we used PartitionFinder with Python v. 2.7 + Anaconda2 (Lanfear et al. 2017). We inferred a Maximum Likelihood phylogenetic tree withIqTree (Nguyen et al. 2015) by using its web server (http://iqtree.cibiv.univie.ac.at/) with the following settings: ultrafast bootstrap method, 1000 bootstrap alignments and nucleotide evolution models of GTR+I+G for the gene 16S and for 1st codon position of COI; GTR+G for RAG1, TYR and 3rd codon position of COI; and GTR for 2nd codon positions of COI. Additionally, we generated a tree using Bayesian Inference using the plug-in MrBayes for Geneious Prime with 1.1 × 10^6 generations and sampling every 200 generations from the Markov Chain Monte Carlo (MCMC). We determined stationarity by plotting the log-likelihood scores of sample points against generation time; when the values reached a stable equilibrium and split frequencies fell below 0.01, stationarity was assumed. We discarded 100,000 samples and 10% of the trees as burn-in. We consider Bayesian Posterior Probabilities (BPP) > 95% as evidence of support for a clade (Huelsenbeck and Ronquist 2001; Wilcox et al. 2002; Aguilar et al. 2013). We visualised both trees in FigTree v.1.4.4.

Finally, we compare uncorrected p-distances of 591 bp (including gaps) of 16S mithocondrial rRNA gene of *Pristimantis* included in our analysis (included as a separated file in: doi: 10.5281/zenodo.8278333). To estimate p-uncorrected genetic distances, we used MEGA 11 (Tamura et al. 2021) with a variance estimation method of 1000 bootstrap and rates amongst sites of G+I. We excluded from this analysis species from the sister clade (*P. bounides*, *P. aniptopalmatus*, *P. albertus*, *P. attenboroughi*, *P. humboldti*, *P. danae*, *P. ornatus*, *P. puipui*, *P. reichlei* and *P. sagittulus*, Fig. 2), except from *Pristimantis* sp.3 from Bolivia because these specimens had been identified as *P. rhabdolaemus* on GenBank and *P. scitulus*, because they are novel sequences for this species.

Quantitative PCR assays for fungal infection (Bd)

During fieldwork in 2018, 2019 and 2021, we swabbed live frogs of the new species with a synthetic dry swab (Medical Wire & Equipment #113) to quantify infection by *Batrachochytrium dendrobatidis* (Bd). We stroked swabs across the skin of juveniles and adults a total of 30 times per individual: five strokes on each side of the abdominal mid-line, five strokes on the inner thighs of each hind leg and five strokes on the foot webbing of each hind leg. We used a standard quantitative Polymerase Chain Reaction (qPCR) assay using DNA extracted from swabs to quantify the level of infection (Boyle et al. 2004). Following the protocol of Boyle et al. (2004) and Hyatt et al. (2007), we extracted DNA from swabs using 40 µl of PrepMan Ultra (Applied Biosystems). We analysed each extract once with a QuantStudio 3 qPCR system (ThermoFisher Scientific). We calculated the number of zoospore equivalents (ZE) (i.e. the genomic equivalent for Bd zoospores) by comparing the sample results with a serial dilution of standards (gBlock synthetic standards, IDT DNA, Iowa, USA). We considered any sample with ZE > 1 to be infected or Bd-positive.

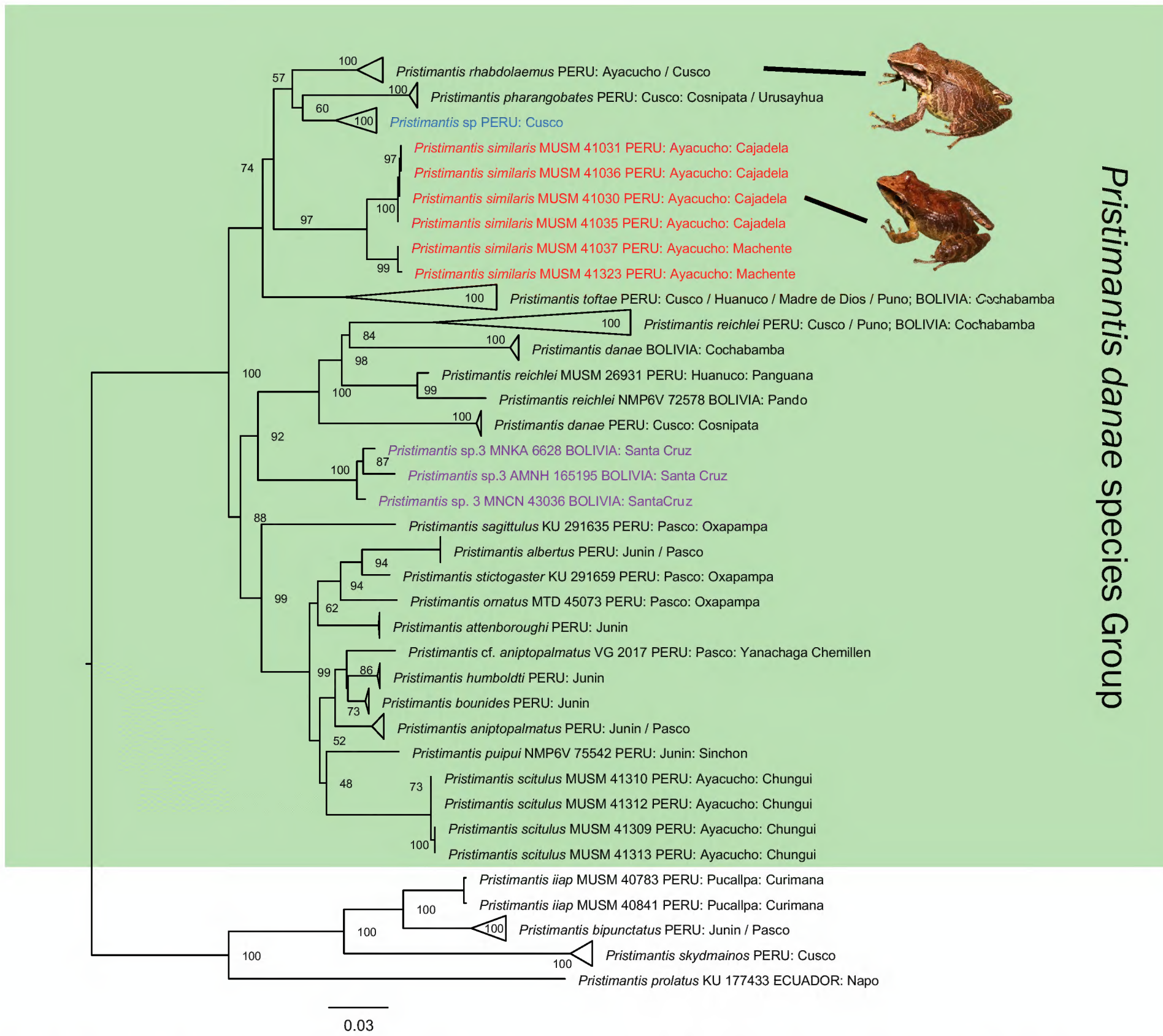
Pristimantis rhabdolaemus PERU: Ayacucho / Cusco
Pristimantis pharangobates PERU: Cusco: Cosnipata / Urusayhua
Pristimantis sp PERU: Cusco
Pristimantis similaris MUSM 41031 PERU: Ayacucho: Cajadela
Pristimantis similaris MUSM 41036 PERU: Ayacucho: Cajadela
Pristimantis similaris MUSM 41030 PERU: Ayacucho: Cajadela
Pristimantis similaris MUSM 41035 PERU: Ayacucho: Cajadela
Pristimantis similaris MUSM 41037 PERU: Ayacucho: Machente
Pristimantis similaris MUSM 41323 PERU: Ayacucho: Machente
Pristimantis toftae PERU: Cusco / Huanuco / Madre de Dios / Puno; BOLIVIA: Cochabamba
Pristimantis reichlei PERU: Cusco / Puno; BOLIVIA: Cochabamba
Pristimantis danae BOLIVIA: Cochabamba
Pristimantis reichlei MUSM 26931 PERU: Huanuco: Panguana
Pristimantis reichlei NMP6V 72578 BOLIVIA: Pando
Pristimantis danae PERU: Cusco: Cosnipata
Pristimantis sp.3 MNKA 6628 BOLIVIA: Santa Cruz
Pristimantis sp.3 AMNH 165195 BOLIVIA: Santa Cruz
Pristimantis sp. 3 MNCN 43036 BOLIVIA: SantaCruz
Pristimantis sagittulus KU 291635 PERU: Pasco: Oxapampa
Pristimantis albertus PERU: Junin / Pasco
Pristimantis stictogaster KU 291659 PERU: Pasco: Oxapampa
Pristimantis ornatus MTD 45073 PERU: Pasco: Oxapampa
Pristimantis attenboroughi PERU: Junin
Pristimantis cf. aniptopalmatus VG 2017 PERU: Pasco: Yanachaga Chemillen
Pristimantis humboldti PERU: Junin
Pristimantis bounides PERU: Junin
Pristimantis aniptopalmatus PERU: Junin / Pasco
Pristimantis puipui NMP6V 75542 PERU: Junin: Sinchon
Pristimantis scitulus MUSM 41310 PERU: Ayacucho: Chungui
Pristimantis scitulus MUSM 41312 PERU: Ayacucho: Chungui
Pristimantis scitulus MUSM 41309 PERU: Ayacucho: Chungui
Pristimantis scitulus MUSM 41313 PERU: Ayacucho: Chungui
Pristimantis iiap MUSM 40783 PERU: Pucallpa: Curimana
Pristimantis iiap MUSM 40841 PERU: Pucallpa: Curimana
Pristimantis bipunctatus PERU: Junin / Pasco
Pristimantis skydmainos PERU: Cusco
Pristimantis prolatus KU 177433 ECUADOR: Napo
0.03
Pristimantis danae species Group

Figure 2. Maximum Likelihood tree of concatenated genes 16S rRNA, COI, RAG1 and TYR taken from GenBank and novel sequences. Numbers on nodes are bootstrap values (see Materials and Methods section for details). Green shadow corresponds to the ingroup. *Pristimantis similaris* sp. nov. in red, *Pristimantis* sp. 3 from Bolivia in purple and *Pristimantis* sp. from Cusco in blue. Maximum Likelihood optimal tree with bootstrap node values from the analysis of a concatenated dataset of 591 bp (16S), 685 bp (COI), 624 bp (RAG1) and 545 bp (TYR) of 128 species aligned by MAFFT and node support assessed using 10,000 replicates in IQTREE.

Nomenclatural acts

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZ) and, hence, the new name contained in the electronic version is effectively published under that Code from the electronic edition alone. This published work and its nomenclatural acts have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information is viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is urn: urn:lsid:zoobank.org:pub:226A24AB-B4BE-4EFD-BF11-D6CA719AB601.

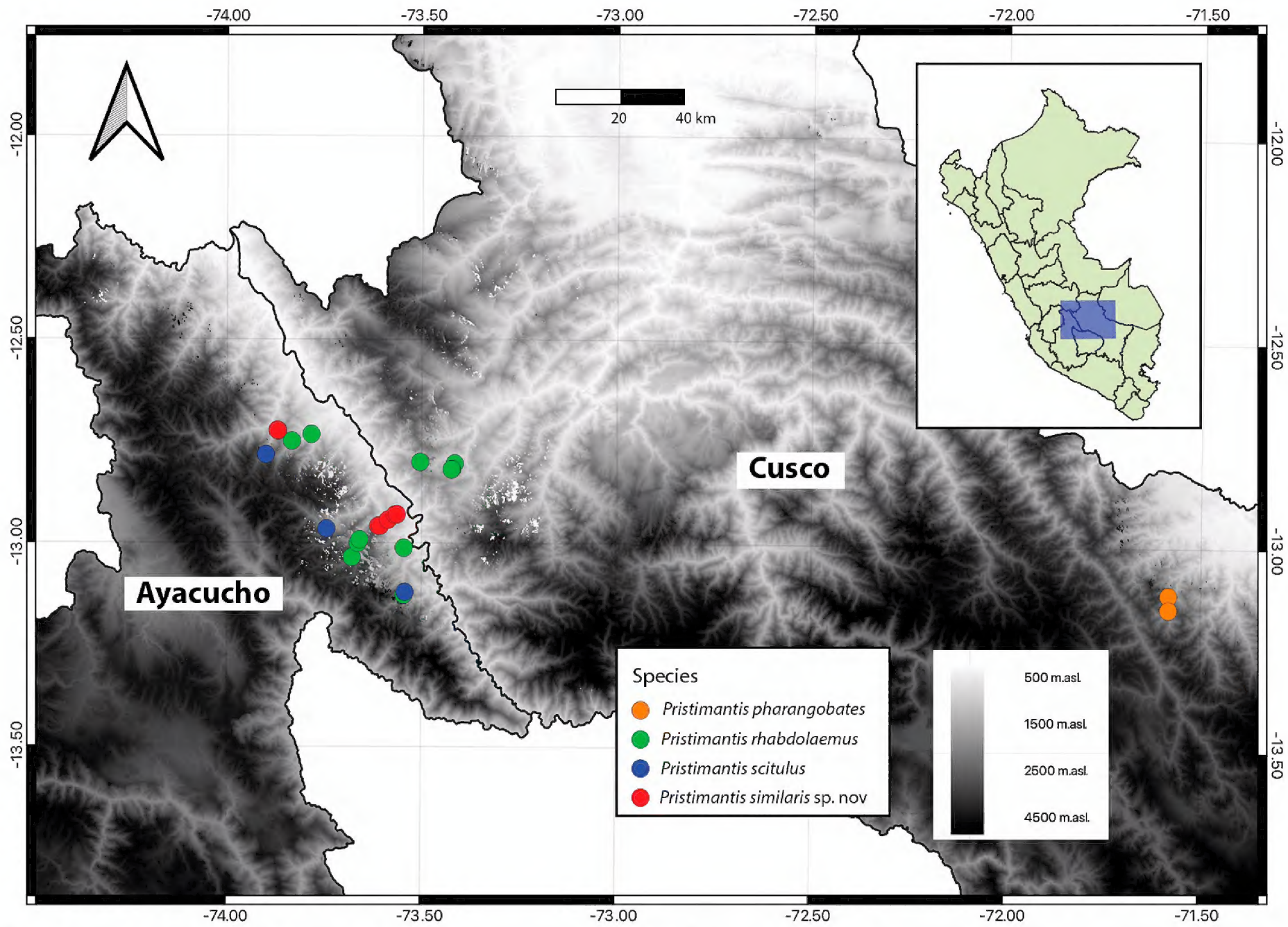

Figure 3. Distribution map of some species of the *Pristimantis danae* species Group in Ayacucho and Cusco Departments. Raster of altitude from 500 to 4500 m. a.s.l. (white to black). Locality of new species in red circles.

Results

Molecular phylogenetic analysis

Our Maximum Likelihood phylogeny, based on four concatenated gene fragments (Fig. 2 and Appendix 3 – expanded ML tree), found the new species (in red) included in a clade with specimens of *Pristimantis rhabdolaemus*, *P. pharangobates* and *P. toftae* from their respective type localities, as well as a candidate species from Cusco (in blue). The results from the Bayesian phylogeny (Appendix 4) are largely congruent with the results from the ML phylogeny.

Pristimantis scitulus is within the *danae* Group and sister to a clade consisting of *P. aniptopalmatus*, *P. humboldti* and *P. bounides*, but with low support. Both our ML and BI phylogenies recover *P. danae* as paraphyletic, with individuals from the type locality in Cosñipata (Cusco, Peru) forming part of a clade that includes *P. danae* specimens from Bolivia and *P. reichlei*, albeit with low support.

Genetic distances (uncorrected p-distances) for the rRNA 16S gene between *P. similaris* sp. nov. and species of the *P. danae* species Group vary from 5.6–6.9% for *P. rhabdolaemus*, 5.9–6.3% for *P. pharangobates*, 6.1–6.7% for *Pristimantis* sp., 6.5–7.5% for *P. scitulus*, 7.3–7.9% for *Pristimantis* sp. 3 and 7.7–9.3% for *P. toftae* (see Suppl. material 1, doi: 10.5281/zenodo.8278333). We also identified two populations within our new species, the first one from the type locality in Cajadela and the second, from Machente. The genetic distances between these populations were 2.7–2.8%.

Fungal infection by *Batrachochytrium dendrobatidis* (Bd)

We found six out of 18 specimens of *P. similaris* (30%) infected by the fungus *Batrachochytrium dendrobatidis* (Bd), with levels of infection varying from 11.5 to 8889.3 zoospore genomic equivalents. Our finding confirms that species of *Pristimantis* can be infected with the fungus (Catenazzi et al. 2011; Warne et al. 2016), despite their life cycle excluding aquatic stages and, thus, limiting the frogs' exposure to the aquatic zoospores of Bd.

Species description

Our phylogenetic tree shows a candidate species from Ayacucho with high support and having high genetic distances with closely-related phylogenetic species (see Fig. 2 and Appendix 3). In addition, after a careful examination of its morphology and pattern of colouration, this candidate species shows differences with other species of the *P. danae* Group. Based on these results, we describe this candidate species from Ayacucho Department as a new species of *Pristimantis*.

***Pristimantis similaris* sp. nov.**
https://zoobank.org/BC56FD8A-6EBD-43C9-A446-689FC3253576
Figs 4–6

Common name. English: Similar Rubber Frog. Spanish: Rana cutín similar.

Generic placement. We assign this species to the genus *Pristimantis*, based on morphology and molecular data (Figs 2, 4, 6).

Type material. *Holotype*. MUSM 41030, adult male (Figs 4, 5) from Comunidad Cajadela (12°57'16.50"S, 73°35'0.70"'W, 1460 m a.s.l.), Distrito Anco, Provincia La Mar, Departamento Ayacucho, Peru, collected on 15 November 2018 by V. Herrera-Alva, E. Castillo-Urbina, V. Díaz, M. Fernandez, and J. Gamboa.

***Paratypes*.** Nine specimens. Five adult females (MUSM 41031, 41032, 41035, 41036 and MUSM 41037). Four adult males (MUSM 41029, 41028, 41033 and MUSM 41034). All the specimens were collected at the type locality, except MUSM 41037, which was collected in Comunidad Machente (12°41'31.70"S, 73°51'0.30"W, 1640 m a.s.l.), Distrito Ayna, Provincia La Mar, Departamento Ayacucho, Peru, on 11 November 2021 by V. Herrera-Alva, E. Castillo-Urbina, V. Díaz and K. Ñaccha.

Diagnosis. A new species of *Pristimantis* assigned to the *P. danae* species Group having the following combination of characters: (1) Skin on dorsum shagreen, skin on venter areolate; discoidal and dorsolateral folds present, weak; thoracic fold present; (2) tympanic membrane and tympanic annulus present, distinct, visible externally; (3) snout subaccuminated in dorsal view, round in lateral view; (4) upper eyelid lacking tubercles; EW smaller than IOD; cranial crest absent; two small and flat tubercles above the snout near the eyes; (5) dentigerous processes of vomers low, oblique in five of the paratypes, absent in four paratypes and the holotype; (6) males with vocal slits, subgular vocal sac large extending on to chest and without nuptial pads; (7) Finger I slightly shorter than Finger II; discs of digits expanded, flat and truncated; (8) fingers without lateral fringes; (9) ulnar tubercles present, but diffuse; (10) heel with

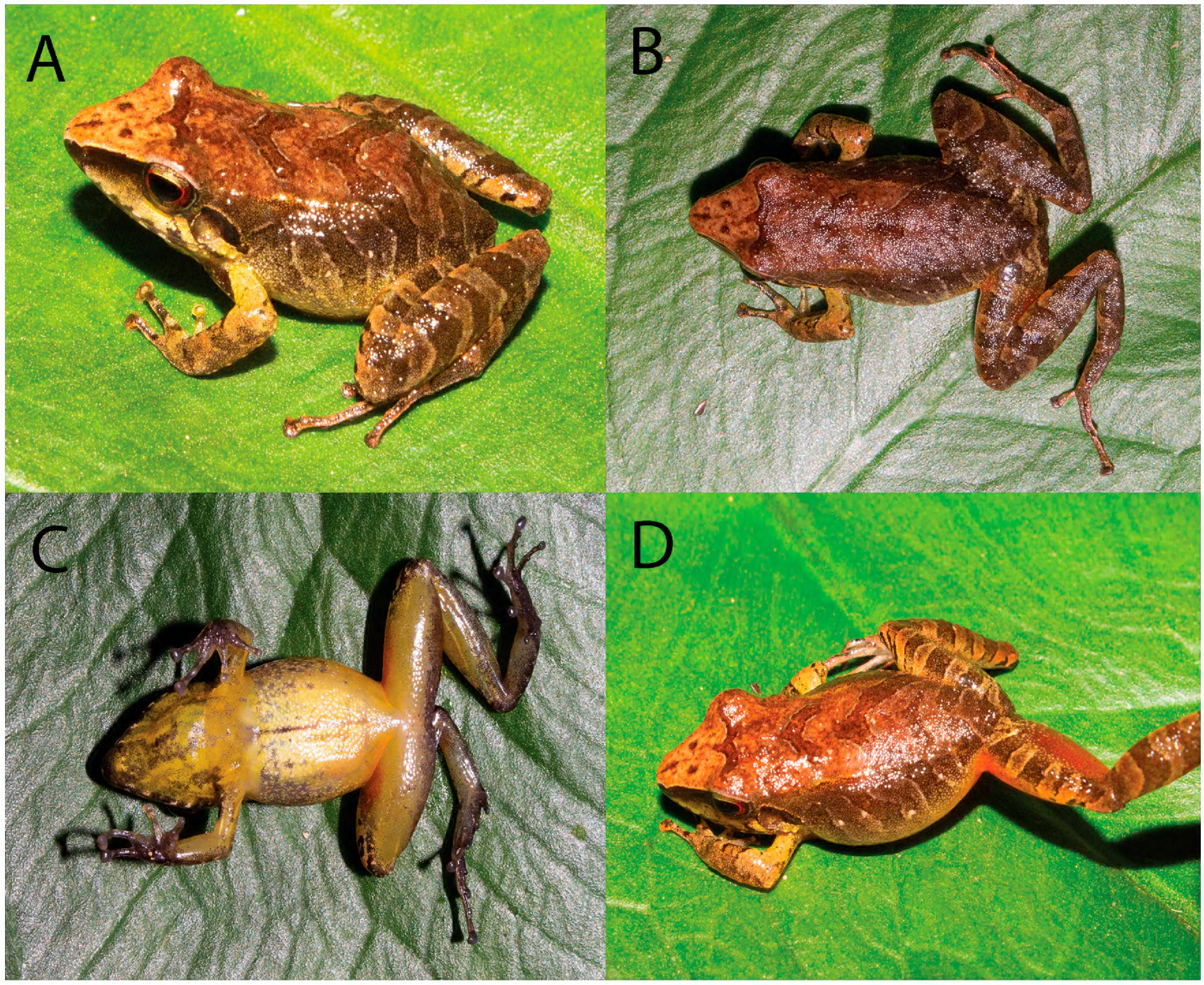

Figure 4. *Pristimantis similaris* sp. nov. (**A–D**) male. SVL: 17.0 mm. Holotype. MUSM 41030. Photos by Vladimir Diaz-Vargas.

two to three small and flat tubercles; inner tarsal fold present, small; (11) inner metatarsal tubercle ovoid, 2–3 times larger than outer one; outer metatarsal tubercle small, ovoid; numerous and flat supernumerary tubercles; (12) toes without lateral fringes; basal toe webbing absent; toe V is slightly longer than toe III; toe discs about as large as those on fingers; (13) in life, dorsum varies from blackish to dark brown with three conspicuous chevrons (not very visible in some cases) (Fig. 6); in most of the adults, the anterior surfaces of thighs reddish-orange, posterior surfaces of thighs brown; flanks cream without tubercles; groin same pattern as flanks mostly, some specimens with orange-reddish colouration (Figs 4, 6); venter cream to yellow with black conspicuous reticulations in the throat and black marks in the chest, males present yellow throat with black or white longitudinal reticulations not as conspicuous as in females (Figs 4, 6); iris dark copper-coloured with fine black vermiculations; (14) SVL in adult females 20.8–25.2 mm (mean = 23.4 ± 1.8 SE, n = 5), in adult males 17.0–18.6 mm (mean = 18.1 mm ± 0.7 SE, n = 5).

Comparisons. *Pristimantis similaris* is distinguished from its congeners in Peru and Bolivia by the following combination of characters: skin on dorsum areolate, tympanum and tympanic annulus distinct, weakly-defined discoidal and dorsolateral folds, two small and flat tubercles above the snout near the

eyes (not conspicuous in preservative), dorsum dark brown with three darker chevrons, anterior surface of thighs usually orange-reddish and posterior surface of thighs dark brown. *Pristimantis similaris* can be distinguished from *P. rhabdolaemus* and *P. pharangobates* by the following characters (characters in parenthesis): smaller SVL of 20.8–25.8 mm in ten females and 15.2–18.9 mm in eight males (*P. pharangobates* 23.1–27.8 mm in females and 15.2–18.2 mm in males; *P. rhabdolaemus* 25.5–31.9 mm in females and 24.1–26.3 mm in males); absence of scapular tubercles (present in both species); presence of conspicuous longitudinal black and white or yellow marks on the throat and chest (less conspicuous in *P. pharangobates* and *P. rhabdolaemus*).

Other species in the *Pristimantis danae* species Group that are similar to *P. similaris* include *P. danae*, *P. reichlei*, *P. scitulus* and *P. toftae*. *Pristimantis danae* and *Pristimantis reichlei* also have brown chevrons in the dorsum and differ from the new species by the combination of the following characters: males nuptial pads absent (present in *P. danae* and *P. reichlei*), dorsolateral folds present (weak in *P. danae* and absent *P. reichlei*), small pale spots in the posterior surfaces of the thighs absent (present in *P. danae* and *P. reichlei*) and smaller size in *P. similaris*. *Pristimantis scitulus* is morphologically similar to *P. similaris* and has a parapatric distribution (Yuraccyacu, in the Piene Valley, Ayacucho Region). *Pristimantis similaris* can be distinguished by lacking a conical tubercle in the upper eyelid and heels (present in *P. scitulus*), mid-ventral line absent (present in *P. scitulus*) and absence of marks in the groin or thighs (conspicuous dark spots in the groin that is continuous as marks on the posterior surfaces of the thighs in *P. scitulus*). *Pristimantis toftae* is a smaller species that is superficially similar to *P. similaris*. The new species can be distinghished by the absence of coloured marks or spots in the groin or other parts of its body (yellow spot in the groins of *P. toftae*), absence of labial bar (presence of a white labial bar in *P. toftae*).

Pristimantis similaris is also similar to some species in the *Pristimantis conspicillatus* species Group, which includes *P. bipunctatus*, *P. skydmainos* and *P. iiap*. The parapatric *Pristimantis bipunctatus* (found in Calicanto at 1940 m. a.s.l. in the Piene Valley, Ayacucho Region), has dorsum and ventral skin shagreen and areolate, snout long, upper eyelids without tubercules similar to *P. similaris*, but the latter differs by having finger I slightly shorter than finger II (finger I and finger II about equal length in *P. bipunctatus*), discs on outer fingers truncated (broadly rounded in *P. bipunctatus*), scapular tubercules absent (present in *P. bipunctatus*) and by its smaller size (22.6–28.8 mm in males and 32.4–41.5 mm in females in *P. bipunctatus*). *P. similaris* can be distinguished from *P. skydmainos* by the absence of a mid-dorsal tubercle (present in *P. skydmainos*), absence of nuptial pads (present in *P. skydmainos*), finger I smaller than finger II (finger I longer than finger II in *P. skydmainos*), absence of spots or marks in the posterior surfaces of the thighs (minute cream flecks on the posterior surfaces of the thighs in *P. skydmainos*) and the absence of W-shaped marks (present in the scapular region in *P. skydmainos*). *Pristimantis similaris* differs from *P. iiap* from the lowland Amazon of the Ucayali Region by lacking large granules on flanks (present in *P. iiap*), lacking granules on the upper eyelids (present in *P. iiap*) and by having finger I shorter than finger II (finger I and II about the same length in *P. iiap*).

Figure 5. *Pristimantis similaris* sp. nov. **A** hand **B** toe. Male. Holotype. MUSM 41030. Photos by VHA.

Another species with some resemblance (mainly on the throat in ventral view) to the new species is *Pristimantis tanyrhynchus*. *Pristimantis similaris* can be distinguished from *P. tanyrhynchus* by the absence of nuptial pads in males (present in *P. tanyrhynchus*) and absence of tubercles on the heel (heel with a conical and large tubercle in *P. tanyrhynchus*).

Description of the holotype. Head longer than wide; head length 43% of SVL; head width 35% of SVL; cranial crests absent; snout subaccuminated in dorsal view and in lateral view (Fig. 4A, B, D); eye-nostril distance same as the eye diameter; nostrils slightly protuberant, directed dorsolaterally; canthus rostralis long, straight in lateral and in dorsal views; loreal region concave; upper eyelid without tubercles, width 90% of IOD (see photo in life Fig. 4); supratympanic fold short and narrow, extending from posterior margin of upper eyelid slightly curved to insertion of arm; tympanic membrane and annulus present; distinct conical postrictal tubercles present bilaterally. Choanae small, ovoid, not concealed by palatal shelf of maxilla; dentigerous processes of vomers absent; vocal slits present; tongue longer than short, oval, about a quarter times as long as wide, notched posteriorly, half of the tongue posteriorly free; one large vocal sac extending on to chest.

Skin on dorsum and flanks shagreen, continuous dorsolateral folds present extending from posterior level of tympanic area to level of hind limb insertion; skin on throat, chest and belly areolate; discoidal fold present; thoracic fold present.

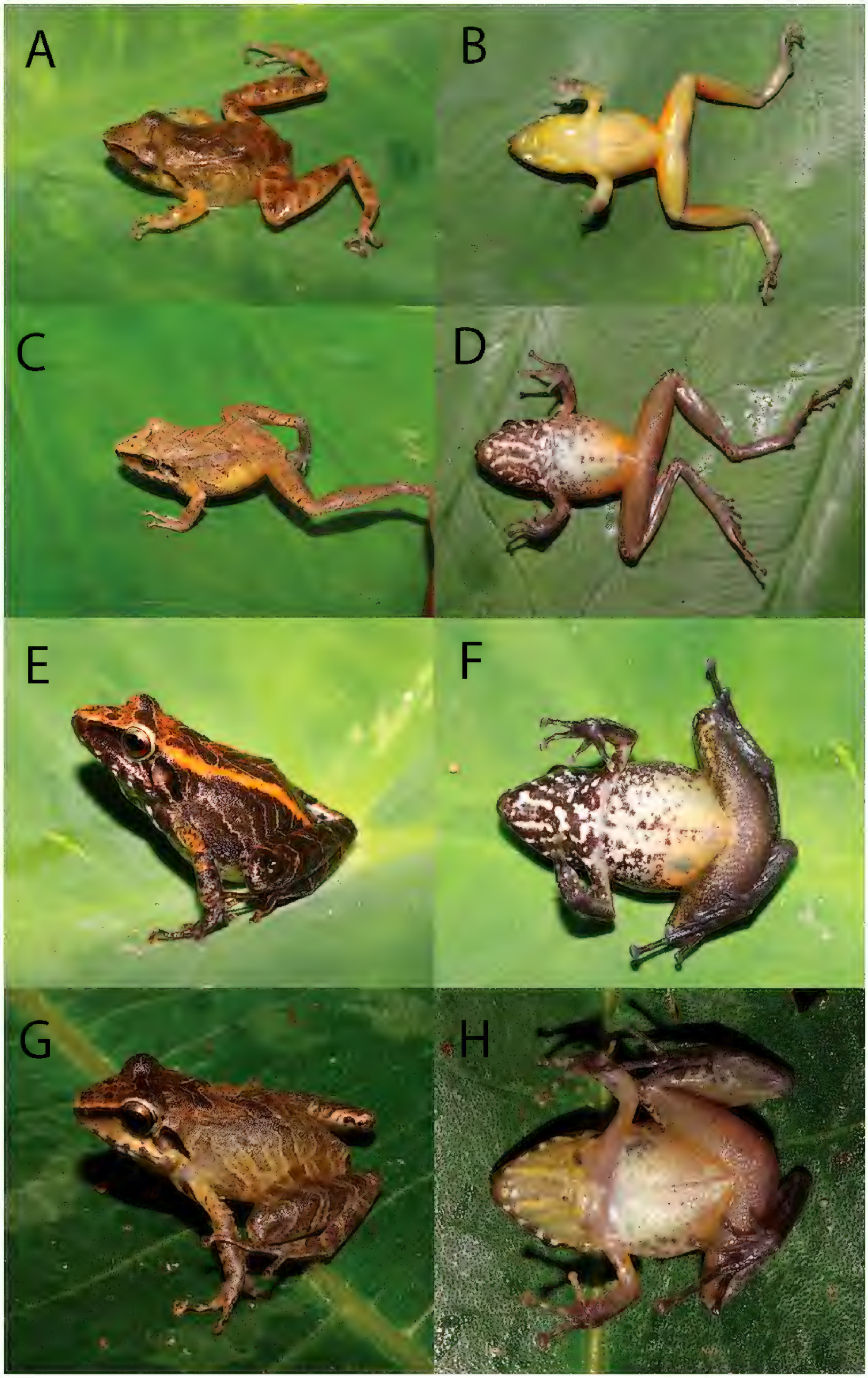

Figure 6. A–H colour and pattern variation of *Pristimantis similaris* sp. nov. Specimen from **A–F** collected in Cajadela: **A, B** male MUSM 41029 **C, D** female MUSM 41031 **E, F** female MUSM 41032. Specimen **G, H** male MUSM 41326 collected in Machente. Photos by V. Diaz-Vargas and E. Castillo-Urbina.

Outer ulnar surface without tubercles; palmar tubercle bifid; thenar tubercle ovoid; subarticular tubercles well defined, most prominent on base of fingers, ovoid in ventral view, subconical in lateral view; supernumerary tubercles indistinct; fingers long and thin lacking lateral fringes, Finger I shorter than Finger II; tips of digits of fingers expanded, truncated, with circumferential grooves; nuptial pads absent (Fig. 5A).

Hind limbs long, slender, tibia length 58% of SVL; foot length 49% of SVL; dorsal surfaces of hind limbs tuberculate; inner surface of thighs smooth, posterior surfaces of thighs shagreen, ventral surfaces of thighs smooth; heels each with three small conical tubercles; outer surface of tarsus with one minute low tubercle; inner tarsal fold present; inner metatarsal tubercle ovoid, two times the size of round outer metatarsal tubercle; subarticular tubercles well defined, ovoid in ventral view, subconical in lateral view; few plantar supernumerary tubercles, about one quarter the size of subarticular tubercles; toes without lateral fringes; basal webbing absent; tips of digits expanded, truncated, less expanded than those on fingers, with circumferential grooves; relative length of toes: 1 < 2 < 3 < 5 < 4; Toe V slightly longer than Toe III (tip of digit of Toe III and Toe V not reaching distal subarticular tubercle on Toe IV; Fig. 5B).

Measurements (in mm) of the holotype. SVL 17.0; tibia length 9.9; foot length 8.4; head length 7.3; head width 5.9; eye diameter 2.3; inter orbital distance 1.9; upper eyelid width 1.7; internarial distance 2.0; eye-nostril distance 2.3; tympanum length 1.0; tympanum height 1.1; forearm length 4.3.

Colouration of the holotype in life (Fig. 4). In life, dorsum dark brown with three conspicuous chevrons; flanks cream without tubercles, groin same pattern as flanks with orange-reddish colouration; anterior surfaces of thighs reddish-orange, posterior surfaces of thighs brown; venter cream to yellow with black conspicuous reticulations in the throat and black marks in the chest; iris dark copper-coloured with fine black vermiculations (Fig. 4).

Colouration of the holotype in preservative. The dorsal ground colouration is pale brown with three browner chevrons; narrow blackish canthal and supratympanic stripes; flanks pale brown with dark brown and cream flecks forming irregularly-shaped diagonal bars; groin and anterior surfaces of thighs brown with dark brown flecks; chest, belly and ventral surfaces of thighs pale cream, throat pale cream and grey-striped; palmar and plantar surfaces and fingers and toes dark brown; iris pale grey.

Variation. All specimens have the same general appearance, with three chevrons on the dorsum. MUSM 41029 is completely yellow and lacks marks on the chest or throat. MUSM 41032 has two brown-yellowish longitudinal bars on the dorsolateral folds. MUSM 41341 is blackish-brown and the three chevrons are not very visible (Fig. 6). Some individuals (MUSM 41030–2, 41036–7) lack the dentigerous processes of vomers. Morphological measurements ranges and proportions of types are included in Tables 2, 3.

Etymology. The specific name corresponds to the Latin word “similar”. This refers to the similarity of the new species and its close phylogenetic relationship with *P. rhabdolaemus* and *P. pharangobates*.

Distribution and natural history. The new species is only known from montane forests of Ayna and Anco in Departamento Ayacucho at elevations from 1200–2000 m a.s.l. in secondary forests (Figs 1, 3). This species was found only at night after 18:00 hours, usually perching on wet leaves 0.5–1.5 m above

Table 2. Morphological measurements (mm) of nine paratype specimens of *Pristimantis similaris* sp. nov. For abbreviations, see Materials and methods.

Character	MUSM 41028	MUSM 41029	MUSM 41031	MUSM 41032	MUSM 41033	MUSM 41034	MUSM 41035	MUSM 41036	MUSM 41037
Sex	Male	Male	Female	Female	Male	Male	Female	Female	Female
SVL	18.1	17.7	25.2	22.7	18.8	18.6	24.7	23.4	20.8
TL	10.3	10.4	14.8	13.5	10.5	10.8	14.0	14.1	12.1
FL	9.0	8.4	12.4	11.3	9.0	9.5	11.6	12.4	9.4
HL	7.2	7.2	9.6	8.9	7.3	7.9	9.8	9.2	8.9
HW	5.9	6.5	8.5	7.5	6.6	6.9	8.2	8.0	7.5
ED	1.9	2.4	2.8	2.5	2.3	2.3	2.9	2.6	2.4
TY	0.9	1.0	1.1	1.1	1.0	1.1	1.3	1.1	1.2
IOD	2.1	2.0	2.4	2.2	2.1	2.0	2.4	2.3	2.2
EW	1.8	1.7	2.3	1.9	1.8	1.8	2.3	2.2	2.1
IND	2.0	2.3	2.9	2.6	2.2	2.2	2.7	2.5	2.5
EN	2.3	2.3	3.1	2.8	2.5	2.6	3.0	2.7	2.8
F4	0.7	0.7	1.0	0.8	0.9	0.7	0.9	1.0	0.8

Table 3. Measurements (in mm) and proportions of adult male and female type specimens of *Pristimantis similaris* sp. nov.; ranges followed by means and one standard deviation in parentheses. For abbreviations, see Materials and methods.

Character	Males (n = 5)	Females (n = 5)
SVL	17.0–18.6 (18.1 ± 0.7)	20.8–25.2 (23.4 ± 1.8)
TL	9.9–10.8 (10.4 ± 0.3)	12.1–14.8 (13.7 ± 1.0)
FL	8.4–9.5 (8.9 ± 0.5)	9.4–12.4 (11.4 ± 1.2)
HL	7.2–7.9 (7.4 ± 0.7)	8.9–9.8 (9.3 ± 0.4)
HW	5.9–6.9 (6.5 ± 1.0)	7.5–8.5 (7.9 ± 0.4)
ED	1.9–2.4 (2.2 ± 0.5)	2.4–2.9 (2.6 ± 0.2)
TY	1.0–1.1 (1.0 ± 0.1)	1.0–1.3 (1.2 ± 0.1)
IOD	1.9–2.1 (2.0 ± 0.1)	2.2–2.4 (2.3 ± 0.1)
EW	1.7–1.8 (1.75 ± 0.05)	1.9–2.3 (2.1 ± 0.2)
IND	2.0–2.3 (2.1 ± 0.1)	2.5–2.9 (2.6 ± 0.2)
EN	2.3–2.6 (2.4 ± 0.3)	2.8–3.1 (2.9 ± 0.2)
F4	0.7–0.9 (0.8 ± 0.1)	0.8–1.0 (0.9 ± 0.1)
TL/SVL	0.56–0.59	0.57–0.60
HL/SVL	0.39–0.43	0.38–0.43
EN/HL	0.32–0.34	0.30–0.33

the ground. Males call rarely and their calls are overshadowed by other male species (*Pristimantis lacrimosus* species Group) calling louder. The species is common and appears to tolerate some human disturbance, because it was found near abandoned farms, less frequented roads and in the sourroudings of abandoned houses. Syntopic species included candidate new species in the *Pristimantis lacrimosus* species Group and candidate new species in the *Pristimantis platydactylus* species Group, which were more abundant than the new

species. Sympatric species included frogs and toads *Gastrotheca pacchamama*, *Nymphargus pluvialis*, *Boana palaestes*, *Rhinella inca*, *Dendropsophus vraemi* and *Hyalinobactrachium* aff. *bergeri*; lizards *Cercosaura manicata*, *Stenocercus crassicaudatus* and *Potamites montanicola*; and snakes *Dipsas* cf. *peruana*, *Leptodeira annulata* and *Epictia* cf. *peruviana*.

Discussion

We describe *Pristimantis similaris*, a new species morphologically similar and phylogenetically related to *P. rhabdolaemus* and *P. pharangobates*. Despite their confusing taxonomic history (see Introduction), our phylogenetic analyses show that *P. rhabdolaemus* and *P. pharangobates* are distinct evolutionary lineages.

Pristimantis rhabdolaemus was described from mid-altitude montane forests restricted to Ayacucho and Cusco Departments (Duellman 1978). Although we visited the type locality of *P. rhabdolaemus* (Machente, Ayacucho Department) at 1650 m a.s.l., we could not find any specimens from this species. For that reason, we sequenced specimens of *P. rhabdolaemus* from Toccate, Anchihuay district in the Ayacucho Department (~ 38 km straight air line to Machente) because morphological analysis and comparison with the type series confirmed that these specimens corresponded to *P. rhabdolaemus sensu stricto*. Padial et al. (2007) reported *P. rhabdolaemus* from Bolivia on the basis of 16S rRNA of two incorrectly assigned specimens (MNKA 6628 and MNCN 43036), but our concatenated phylogeny suggests that the Bolivian specimens belong to a different and probably undescribed species, *Pristimantis* sp. 3 (in purple, Fig. 2). Likewise, *P. pharangobates* should be restricted to Cusco Department until molecular data become available and support the presence of this species in Puno (south-eastern Peru) and Bolivia.

We also found another candidate species from Cosñipata and Alfamayo in Cusco, morphologically similar and phylogenetically related to *P. pharangobates* and *P. rhabdolaemus* (in blue, Fig. 2). Additional specimens and analyses are needed to assess the taxonomic status of these potential new species.

The taxonomy of other species of the *P. danae* species Group requires further work. For instance, specimens identified as *P. danae* or *P. reichlei* tend to form paraphyletic groups in phylogenies. We suggest that both species might benefit from future studies clarifying the phylogenetic relationships of their assigned populations. Such studies might include the use of genomic data for these species (including *P. toftae*) because the use of four genes (three nuclear) in this study was not sufficiently informative to infer with confidence phylogenetic relationships between the most inclusive clades.

Furthermore, our phylogenies include for the first time sequences of *P. scitulus* from Chungui, Ayacucho previously known only from two type specimens in Yuraccyacu, Ayacucho (at 2600 m a.s.l) and supports the assignment of this species in the *P. danae* species Group. We also included sequences for the first time of *P. iiap* (outgroup) and it is recovered in the *P. conspicillatus* species Group.

According to Swenson et al. (2012), who discussed the endemism of species (birds, mammals, plants and amphibians) in the eastern Andean slopes from the treeline (~ 3500 m a.s.l.) to the Amazon lowlands, most of the montane forests in the eastern Andean slopes of Peru and Bolivia (fig. 7

in Swenson et al. (2012)) are centres of endemism, specially in areas with little field evaluations due to social problems, such as montane forests in Ayacucho Department.

We would like to highlight the areas surroundings the type locality of *P. similaris* and closely-related species in south-eastern Peru. The Departments of Ayacucho and Cusco have biologically "irreplaceable areas" due to the configuration of the western Andes, the eastern Cordillera de Vilcabamba and the Apurimac River (Swenson et al. 2012). These geographical formations created a deep canyon along the Apurimac River at the border of the Departments of Ayacucho and Cusco (Lehr and Catenazzi 2008; Hazzi et al. 2018; Herrera-Alva et al. 2020), dissecting the Andean cordillera and providing mid-altitude isolated areas. The Apurimac Canyon is an important barrier for the dispersal of amphibians, such as high-altitude species of Terrarana: the Canyon splits the distribution of the genus *Phrynopus* to the northeast part of the Canyon from the distribution of *Bryophryne* southwest of the Canyon (Lehr and Catenazzi 2008, 2009, 2010). We believe that this pattern can be extended to mid-altitude montane forest frogs, such as species in the *P. danae* or *P. lacrimosus* species groups (pers. com. Ernesto Castillo-Urbina) or the distribution of mid-altitude toads, such as *Atelopus moropukaqumir* (northwest of the Apurimac Canyon) and *A. erythropus* (southeast of the Canyon; Herrera-Alva et al. (2020)). Therefore, we hypothesise that the Apurimac Canyon could have promoted vicariant speciation of morphologically-similar *Pristimantis* in these montane forests. For instance, *P. similaris* occurs from 1200 to 2000 m a.s.l. on the northwest of the Apurimac Canyon in Ayacucho Department, while *Pristimantis* sp. ocurrs from 1200–2000 m a.s.l. in Cusco Department, southeast of the Apurimac Canyon. Nevertheless, the Apurimac Canyon might have not been a geographic barrier to other species, such as *P. rhabdolaemus* which has been found at both sides of the canyon. One population of this species has an altitude range from 2000–2900 m a.s.l. in Ayacucho (eastern part of the Apurimac River) and the other population ranges from 2000–2100 m a.s.l. in Cusco (western part of the Apurimac River) according to available specimens and sequences. The presence of *P. rhabdolaemus* on both sides of the Apurimac River will remain hypothetical until more specimens and tissues from Cusco Department become available and are tested against a hypothesis of two different species following an integrative approach.

We also provide information about infection by the fungus *Batrachochytrium dendrobatidis* (Bd). Chytridiomycosis, caused by the Bd fungus, has negatively affected amphibian communities in the montane forests of Central America and South America (Berger et al. 1998; Lips et al. 2008; Catenazzi et al. 2011; Catenazzi 2015). This pathogen has been associated with amphibian worldwide declines (Berger et al. 1998; Briggs et al. 2005; Lips et al. 2006; Catenazzi et al. 2010; Scheele et al. 2019). Catenazzi et al. (2011) reported a rapid decline in frog species richness and abundance from 1999 to 2008 in the upper Manu National Park (Cusco), which has communities and ecosystems similar to those found in our study area (Ayacucho). The high prevalence of 30% in *P. similaris* suggests that Bd could be threatening amphibians in the area and that Bd transmission (which is typically associated with aquatic species, given that the infective zoopores are aquatic) occurs in terrestrial frogs.

Acknowledgements

Fieldwork was possible by the Vicerrectorado de Investigación y Posgrado de la Universidad Nacional Mayor de San Marcos (award project code B22100451 to CAP and VHA) and Prociencia - Concytec for their financial support under contract PE501081904-2022 to VHA. Furthermore, we thank Ernesto Castillo-Urbina, Vladimir Díaz, Kimberly Ñaccha, Maura Fernandez and Juan Gamboa for their support in the field collecting the specimens; and to Sebastián Riva-Regalado for the final editions in the photos of this manuscript. We thank Anna Motta and Rafe Brown from KU, Pablo Venegas and Juan Carlos Chavez from CORBIDI and Juan Carlos Chaparro and Maria Isabel Diaz from MUBI for their help and guidance in the process of reviewing museum specimens. Finally, we thank Edgar Lehr, Karen Siu Ting and Rudolf von May for their valuable reviews and comments that improved the quality of our manuscript.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

Funding from Vicerrectorado de Investigación y Posgrado (UNMSM) B22100451 was provided to CAP and VHA, and Prociencia – Concytec PE501081904-2022 to VHA.

Author contributions

Writing - original draft: VHA. Writing - review and editing: VHA, AC, CAP. Investigation: VHA, AC, CAP. Methodology: VHA, AC, CAP. Funding acquisition: CAP, VHA. Data curation: VHA. Formal analysis: VHA, AC. DNA Sequencing: AC. Thesis advice: CAP, AC.

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Data availability

All of the data that support the findings of this study are available in the main text.

References

Acevedo AA, Armesto O, Palma E (2020) Two new species of *Pristimantis* (Anura: Craugastoridae) with notes on the distribution of the genus in northeastern Colombia. Zootaxa 4750: 499–523. https://doi.org/10.11646/zootaxa.4750.4.3

Aguilar C, Wood Jr PL, Cusi JC, Guzman A, Huari F, Lundberg M, Mortensen E, Ramirez C, Robles D, Suarez J, Ticona A, Vargas VJ, Venegas PJ, Sites Jr JW (2013) Integrative taxonomy and preliminary assessment of species limits in the *Liolaemus walkeri*

complex (Squamata, Liolaemidae) with descriptions of three new species from Peru. ZooKeys 47(364): 47–91. https://doi.org/10.3897/zookeys.364.6109

Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, Scolombe R, Ragan MA, Hyatt AD, McDonald K, Hines HB, Lips KR, Maranteli G, Parkes H (1998) Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proceedings of the National Academy of Sciences of the United States of America 95(15): 9031–9036. https://doi.org/10.1073/pnas.95.15.9031

Bossuyt F, Milinkovitch MC (2000) Convergent adaptive radiations in Madagascan and Asian ranid frogs reveal covariation between larval and adult traits. Proceedings of the National Academy of Sciences of the United States of America 97: 6585–6590. https://doi.org/10.1073/pnas.97.12.6585

Boyle DG, Boyle DB, Olsen V, Morgan JAT, Hyatt AD (2004) Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. Diseases of Aquatic Organisms 60(2): 141–148. https://doi.org/10.3354/dao060141

Briggs JM, Knapp AK, Blair JM, Heisler JL, Hoch GA, Lett MS, McCarron JK (2005) An ecosystem in transition: Causes and consequences of the conversion of mesic grassland to shrubland. Bioscience 55(3): 243–254. https://doi.org/10.1641/0006-3568(2005)055[0243:AEITCA]2.0.CO;2

Carrión-Olmedo JC, Ron SR (2021) A new cryptic species of the *Pristimantis lacrimosus* group (Anura, Strabomantidae) from the eastern slopes of the Ecuadorian Andes. Evolutionary Systematics 5: 151–175. https://doi.org/10.3897/evolsyst.5.62661

Catenazzi A (2015) State of the world's amphibians. Annual Review of Environment and Resources 40(1): 91–119. https://doi.org/10.1146/annurev-environ-102014-021358

Catenazzi A, Vredenburg VT, Lehr E (2010) *Batrachochytrium dendrobatidis* in the live frog trade of Telmatobius (Anura: Ceratophryidae) in the tropical Andes. Diseases of Aquatic Organisms 92(2–3): 187–191. https://doi.org/10.3354/dao02250

Catenazzi A, Lehr E, Rodriguez LO, Vredenburg VT (2011) *Batrachochytrium dendrobatidis* and the collapse of anuran species richness and abundance in the upper Manu National Park, southeastern Peru. Conservation Biology 25(2): 382–391. https://doi.org/10.1111/j.1523-1739.2010.01604.x

Dayrat B (2005) Towards integrative taxonomy. Biological Journal of the Linnean Society, Linnean Society of London 85(3): 407–417. https://doi.org/10.1111/j.1095-8312.2005.00503.x

De Queiroz K (2005) Ernst Mayr and the modern concept of species. Proceedings of the National Academy of Sciences 102(suppl_1): 6600–6607. https://doi.org/10.1073/pnas.0502030102

Duellman WE (1978) Two New Species of *Eleutherodactylus* (Anura: Leptodactylidae) from the Peruvian Andes. Transactions of the Kansas Academy of Science (1903) 81(1): 65–71. https://doi.org/10.2307/3627358

Duellman WE, Lehr E (2009) Terrestrial-breeding frogs (Strabomantidae) in Peru. Natur und Tier Verlag.

Duellman WE, Lehr E, Venegas PJ (2006) Two new species of *Eleutherodactylus* (Anura: Leptodactylidae) from the Andes of northern Peru. Zootaxa 1285(1): 51–64. https://doi.org/10.11646/zootaxa.1512.1.3

Elmer KR, Cannatella DC (2008) Three new species of leaflitter frogs from the upper Amazon forests: cryptic diversity within *Pristimantis ockendeni* (Anura: Strabomantidae) in Ecuador. Zootaxa 1784(1): 11–38. https://doi.org/10.11646/zootaxa.1784.1.2

Faivovich J, Haddad CF, Garcia PC, Frost DR, Campbell JA, Wheeler WC (2005) Systematic review of the frog family Hylidae, with special reference to Hylinae: Phylogenetic analysis and taxonomic revision. Bulletin of the American Museum of Natural History 2005(294): 1–240. https://doi.org/10.1206/0003-0090(2005)294[0001:SROTFF]2.0.CO;2

Frost DR (2023) Amphibian Species of the World: an Online Reference. Version 6.2 (30 March 2023). Electronic Database accessible at https://amphibiansoftheworld.amnh.org/index.php. American Museum of Natural History, New York, USA. https://doi.org/10.5531/db.vz.0001

Guayasamin JM, Hutter CR, Tapia EE, Culebras J, Peñafiel N, Pyron RA, Morochz C, Funk WC, Arteaga A (2017) Diversification of the rainfrog *Pristimantis ornatissimus* in the lowlands and Andean foothills of Ecuador. PLoS ONE 12(3): e0172615. https://doi.org/10.1371/journal.pone.0172615

Hazzi NA, Moreno JS, Ortiz-Movliav C, Palacio RD (2018) Biogeographic regions and events of isolation and diversification of the endemic biota of the tropical Andes. Proceedings of the National Academy of Sciences of the United States of America 115(31): 7985–7990. https://doi.org/10.1073/pnas.1803908115

Hedges SB, Duellman WE, Heinicke MP (2008) New World direct-developing frogs (Anura: Terrarana): molecular phylogeny, classification, biogeography, and conservation. Zootaxa 1737(1): 1–182. https://doi.org/10.11646/zootaxa.1737.1.1

Heinicke MP, Duellman WE, Hedges SB (2007) Major Caribbean and Central American frog faunas originated by ancient oceanic dispersal. Proceedings of the National Academy of Sciences of the United States of America 104(24): 10092–10097. https://doi.org/10.1073/pnas.0611051104

Herrera-Alva V, Díaz V, Castillo-Urbina E, Rodolfo C, Catenazzi A (2020) A new species of *Atelopus* (Anura: Bufonidae) from southern Peru. Zootaxa 4853(3): 404–420. https://doi.org/10.11646/zootaxa.4853.3.4

Hillis DM (2019) Species delimitation in herpetology. Journal of Herpetology 53(1): 3–12. https://doi.org/10.1670/18-123

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17(8): 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Hutter CR, Guayasamin JM (2015) Cryptic diversity concealed in the Andean cloud forests: Two new species of rainfrogs (*Pristimantis*) uncovered by molecular and bioacoustic data. Neotropical Biodiversity 1(1): 36–59. https://doi.org/10.1080/23766808.2015.1100376

Hyatt AD, Boyle DG, Olsen V, Boyle DB, Berger L, Obendorf D, Dalton A, Kriger K, Hero M, Hines H, Phillott R, Campbell R, Marantelli G, Gleason F, Colling A (2007) Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. Diseases of Aquatic Organisms 73: 175–192. https://doi.org/10.3354/dao073175

Kieswetter CM, Schneider CJ (2013) Phylogeography in the northern Andes: Complex history and cryptic diversity in a cloud forest frog, *Pristimantis w-nigrum* (Craugastoridae). Molecular Phylogenetics and Evolution 69(3): 417–429. https://doi.org/10.1016/j.ympev.2013.08.007

Köhler J, Castillo-Urbina E, Aguilar-Puntriano C, Vences M, Glaw F (2022) Rediscovery, redescription and identity of *Pristimantis nebulosus* (Henle, 1992), and description of a new terrestrial-breeding frog from montane rainforests of central Peru (Anura, Strabomantidae). Zoosystematics and Evolution 98(2): 213–232. https://doi.org/10.3897/zse.98.84963

Lanfear R, Calcott B, Ho SYW, Guindon S (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molecular Biology and Evolution 29: 1695–1701. https://doi.org/10.1093/molbev/mss020

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B (2017) PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Molecular Biology and Evolution 34(3): 772–773. https://doi.org/10.1093/molbev/msw260

Lehr E (2007) New eleutherodactyline frogs (Leptodactylidae: *Pristimantis*, *Phrynopus*) from Peru. Bulletin of the Museum of Comparative Zoology 159(2): 145–178. https://doi.org/10.3099/0027-4100(2007)159[145:NEFLPP]2.0.CO;2

Lehr E, Catenazzi A (2008) A new species of *Bryophryne* (Anura: Strabomantidae) from southern Peru. Zootaxa 1784(1): 1–10. https://doi.org/10.11646/zootaxa.1784.1.1

Lehr E, Catenazzi A (2009) Three new species of *Bryophryne* (Anura: Strabomantidae) from the region of Cusco, Peru. South American Journal of Herpetology 4(2): 125–138. https://doi.org/10.2994/057.004.0204

Lehr E, Catenazzi A (2010) Two new species of *Bryophryne* (Anura: Strabomantidae) from high elevations in southern Peru (Region of Cusco). Herpetologica 66(3): 308–319. https://doi.org/10.1655/09-038.1

Lehr E, von May R (2017) A new species of terrestrial-breeding frog (Amphibia, Craugastoridae, *Pristimantis*) from high elevations of the Pui Pui Protected Forest in central Peru. ZooKeys 17(660): 17–42. https://doi.org/10.3897/zookeys.660.11394

Lehr E, Moravec J, Cusi JC, Gvoždík V (2017a) A new minute species of *Pristimantis* (Amphibia: Anura: Craugastoridae) with a large head from the Yanachaga-Chemillén National Park in central Peru, with comments on the phylogenetic diversity of *Pristimantis* occurring in the Cordillera Yanachaga. European Journal of Taxonomy 325: 1–22. https://doi.org/10.5852/ejt.2017.325

Lehr E, Von May R, Moravec J, Cusi JC (2017b) Three new species of *Pristimantis* (Amphibia, Anura, Craugastoridae) from upper montane forests and high Andean grasslands of the Pui Pui Protected Forest in central Peru. Zootaxa 4299(3): 301–336. https://doi.org/10.11646/zootaxa.4299.3.1

Lips KR, Brem F, Brenes R, Reeve JD, Alford RA, Voyles J, Carey C, Livo L, Pessier A, Collins JP (2006) Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. Proceedings of the National Academy of Sciences of the United States of America 103(9): 3165–3170. https://doi.org/10.1073/pnas.0506889103

Lips KR, Diffendorfer J, Mendelson JR III, Sears MW (2008) Riding the wave: Reconciling the roles of disease and climate change in amphibian declines. PLoS Biology 6(3): e72. https://doi.org/10.1371/journal.pbio.0060072

Lynch JD, Duellman WE (1997) Frogs of the genus *Eleutherodactylus* (Leptodactylidae) in western Ecuador: systematic, ecology, and biogeography. Natural History Museum, University of Kansas, 252 pp. https://doi.org/10.5962/bhl.title.7951

Lynch JD, McDiarmid R (1987) Two new species of *Eleutherodactylus* (Amphibia: Anura: Leptodactylidae) for Bolivia. Proceedings of the Biological Society of Washington 100: 337–346. https://doi.org/10.2307/1447850

Meyer CP (2003) Molecular systematics of cowries (Gastropoda: Cypraeidae) and diversification patterns in the tropics. Biological Journal Linnean Society 79: 401–459. https://doi.org/10.1046/j.1095-8312.2003.00197.x

Minoli I, Morando M, Avila LJ (2014) Integrative taxonomy in the *Liolaemus fitzingerii* complex (Squamata: Liolaemini) based on morphological analyses and niche modeling. Zootaxa 3856(4): 501–528. https://doi.org/10.11646/zootaxa.3856.4.3

Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300

Ortega-Andrade HM, Rojas-Soto OR, Valencia JH, Espinosa de los Monteros A, Morrone JJ, Ron SR, Cannatella DC (2015) Insights from integrative systematics reveal cryptic diversity in *Pristimantis* frogs (Anura: Craugastoridae) from the Upper Amazon Basin. PLoS ONE 10(11): e0143392. https://doi.org/10.1371/journal.pone.0143392

Padial JM, De la Riva I (2009) Integrative taxonomy reveals cryptic Amazonian species of *Pristimantis* (Anura: Strabomantidae). Zoological Journal of the Linnean Society 155(1): 97–122. https://doi.org/10.1111/j.1096-3642.2008.00424.x

Padial JM, Castroviejo-Fisher S, Köhler J, Domic E, De la Riva I (2007) Systematics of the *Eleutherodactylus fraudator* species group (Anura: Brachycephalidae). Herpetological Monograph 21(1): 213–240. https://doi.org/10.1655/06-007.1

Padial JM, Taran G, Frost DR (2014) Molecular systematics of terraranas (Anura: Brachycephaloidea) with an assessment of the effects of alignment and optimality criteria. Zootaxa 3825(1): 1–132. https://doi.org/10.11646/zootaxa.3825.1.1

Páez NB, Ron SR (2019) Systematics of Huicundomantis, a new subgenus of *Pristimantis* (Anura, Strabomantidae) with extraordinary cryptic diversity and eleven new species. ZooKeys 868: 1–112. https://doi.org/10.3897/zookeys.868.26766

Pinto-Sánchez NR, Ibáñez R, Madriñán S, Sanjur OI, Bermingham E, Crawford AJ (2012) The great American biotic interchange in frogs: multiple and early colonization of Central America by the South American genus *Pristimantis* (Anura: Craugastoridae). Molecular Phylogenetics and Evolution 62(3): 954–972. https://doi.org/10.1016/j.ympev.2011.11.022

Pyron RA, Wiens JJ (2011) A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. Molecular Phylogenetics and Evolution 61(2): 543–583. https://doi.org/10.1016/j.ympev.2011.06.012

Reyes-Puig C, Mancero E (2022) Beyond the species name: An analysis of publication trends and biases in taxonomic descriptions of rainfrogs (Amphibia, Strabomantidae, *Pristimantis*). ZooKeys 1134: 73–100. https://doi.org/10.3897/zookeys.1134.91348

Rojas RR, Fouquet A, Ron SR, Hernández-Ruz EJ, Melo-Sampaio PR, Chaparro JC, Vogt RC, Tadeus De Carvalho V, Cardoso Pinheiro L, Avila R, Pires A, Gordo M, Hrbek T (2018) A Pan-Amazonian species delimitation: high species diversity within the genus *Amazophrynella* (Anura: Bufonidae). PeerJ 6: e4941. https://doi.org/10.7717/peerj.4941

Scheele BC, Pasmans F, Berger L, Skerratt LF, Martel A, Beukema W, Acevedo AA, Burrowes PA, Carvhalo T, Catenazzi A, De la Riva I, Fisher MC, Flechas S, Foster C, Frías-Alvarez P, Garner TWJ, Gratwicke B, Guayasamin JM, Hirschfeld M, Kolby JE, Kosch TA, La Marca E, Lindenmayer DB, Lips KR, Longo AV, Maneyro R, McDonald CA, Mendelson J, Palacios-Rodriguez P, Parra-Olea G, Richards-Zawacki CL, Rödel M, Rovito SM, Soto-Azat C, Toledo LF, Voyles J, Weldon C, Whitfield SM, Wilkinson M, Zamudio KR, Canessa S (2019) The aftermath of an amphibian fungal panzootic reveals unprecedented loss of biodiversity. Science 636: 1459–1463. https://doi.org/10.1126/science.aav0379

Schlick-Steiner BC, Steiner FM, Seifert B, Stauffer C, Christian E, Crozier RH (2010) Integrative taxonomy: A multisource approach to exploring biodiversity. Annual Review of Entomology 55(1): 421–438. https://doi.org/10.1146/annurev-ento-112408-085432

Simpson GG (1961) Principles of animal taxonomy. In Principles of Animal Taxonomy. Columbia University Press, 250 pp. https://doi.org/10.7312/simp92414

Siqueira S, Aguiar Jr O, Souza MB, Lima AP, Recco-Pimentel SM (2009) Unusual intra-individual karyotypical variation and evidence of cryptic species in Amazonian populations of *Pristimantis* (Anura, Terrarana). Hereditas 146(4): 141–151. https://doi.org/10.1111/j.1601-5223.2009.02104.x

Swenson JJ, Young BE, Beck S, Comer P, Córdova JH, Dyson J, Embert D, Encarnación F, Ferreira W, Franke I, Grossman D, Hernadez P, Herzog SK, Josse C, Navarro G, Pacheco V, Stein BA, Timaná M, Tovar A, Tovar C, Vargas J, Zambrana-Torrelio CM (2012) Plant and animal endemism in the eastern Andean slope: Challenges to conservation. BMC Ecology 12(1): 1–19. https://doi.org/10.1186/1472-6785-12-1

Tamura K, Glen S, Sudhir K (2021) MEGA11: Molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution 38(7): 3022–3027. https://doi.org/10.1093/molbev/msab120

von May R, Catenazzi A, Corl A, Santa-Cruz R, Carnaval AC, Moritz C (2017) Divergence of thermal physiological traits in terrestrial breeding frogs along a tropical elevational gradient. Ecology and Evolution 7(9): 3257–3267. https://doi.org/10.1002/ece3.2929

Waddell EH, Crotti M, Lougheed SC, Cannatella DC, Elmer KR (2018) Hierarchies of evolutionary radiation in the world's most species rich vertebrate group, the Neotropical *Pristimantis* leaf litter frogs. Systematics and Biodiversity 16(8): 807–819. https://doi.org/10.1080/14772000.2018.1503202

Warne RW, LaBumbard B, LaGrange S, Vredenburg VT, Catenazzi A (2016) Co-Infection by Chytrid Fungus and Ranaviruses in Wild and Harvested Frogs in the Tropical Andes. PLoS ONE 11(1): e0145864. https://doi.org/10.1371/journal.pone.0145864

Wilcox TP, Zwickl DJ, Heath TA, Hillis DM (2002) Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. Molecular Phylogenetics and Evolution 25: 361–371. https://doi.org/10.1016/S1055-7903(02)00244-0

Wiley EO (1978) The evolutionary species concept reconsidered. Systematic Zoology 27(1): 17–26. https://doi.org/10.2307/2412809

Zozaya SM, Higgie M, Moritz C, Hoskin CJ (2019) Are pheromones key to unlocking cryptic lizard diversity? American Naturalist 194(2): 168–182. https://doi.org/10.1086/704059

Appendix 1

Specimens analysed from museums. See acronyms in Materials and methods. Underlined codes correspond to type material. Coordinates and altitude when available.

***Pristimantis pharangobates*:** AMNH 6099, 153089: Between Abra Accanaco and Pillahuata, Paucartambo, Cusco; KU 173236–173251: Buenos Aires, Cosñipata, Paucartambo, Cusco [-13.15; -71.5833; altitude: 2400 m a.s.l.]; MUBI 4203, 4205, 4209–10, 4212, 4217, 4220, 4222, 4224–4225, 4228, 4390–92, 4395–97, 4399–4400, 4560–61, 4563, 4610: Cosñipata, Paucartambo, Cusco [-13.1153; -715833; altitude: 2750 m a.s.l.]; MUBI 11451–52, 11374–87: Trocha Unión, Cosñipata, Paucartambo, Cusco [-13.1061; -71.5897; altitude: 2780 m a.s.l.]; MUSM 27910: Buenos Aires, Cosñipata, Paucartambo, Cusco; MUSM 32932–35, 32952–60: Paucartambo, Cusco.

***Pristimantis rhabdolaemus*:** CORBIDI 10813, 10815–16: Chiquintirca, La Mar, Ayacucho [-13.0350; -73.6786; altitude: 2635 m a.s.l.]; LSUMZ 26150–51, 26153, 26156, 26182, 26251: Huanhuachayocc, Tambo to Apurimac Road [-12.75; -73.7833]; KU 175082–84: Huanhuachayocc, Tambo to Apurimac Road [-12.73; -73.7833]; MUBI 17541–42, 17552, 17555: Aendoshari Community, La Convención, Cusco [-12.8188; -73.4239; altitude: 2350 m a.s.l.];

MUSM 18505-08: Toccate, La Mar, Ayacucho [-12.9950; -73.6685; altitude: 2080 m a.s.l.]; 30206–08: Cielo Punku, Kimbiri, La Convención, Cusco [-12.8008; -73.5042; altitude: 2100 m a.s.l.]; KNC 44–45, 47–48, 106, 116, 118–20, 122, 130: Chaupichaca, Chungui, La Mar, Ayacucho [-13.1219; -73.5439; altitude: 2040–2345 m a.s.l.].

***Pristimantis scitulus*:** KU 175085: Yuraccyacu, Tambo to Apurimac Road; LSUMZ 26097: Yuraccyacu, Tambo to Apurimac Road [-12.7833; -73.9]; MUSM 41309-10, 41312-13: Chaupichaca, Chungui, La Mar, Ayacucho [-13.12; -73.5228; altitude: 2270–2520 m a.s.l.].

***Pristimantis* sp.:** AMNH 153088, 153090: Between Accanaco and Pillahuata, Paucartambo, Cusco; KU 138877: 7 km WSW Santa Isabel, Cosñipata, Paucartambo, Cusco; MUBI 13317, 13323–24, 13333–34: San Pedro, Paucartambo, Cusco; MUSM 21035, 26269–70, 26276, 30418, 30433, 32934: Suecia, Paucartambo, Cusco. KU 175086–88: Huyro, Quillabamba, Echarate, Cusco; MUBI 13612, 13627, 13661: Mesa Pelada, Huayopata, La Convención, Cusco; MUSM 27911–12: Alfamayo, Huayopata, La Convención, Cusco.

***Pristimantis* sp. 4.:** LSUMZ 26147–48, 26154, 26158: Between Pataccocha and San Jose, Santa Rosa, Ayna, Ayacucho.

Appendix 2

Table A1. Sequences used from GenBank and new sequences added in this paper.

N°	Species	Voucher	Group	Locality	Source	16 S	COI	RAG1	TYR
1	*Pristimantis albertus*	KU 291675	Ingroup	Peru: Pasco, 0.9 km N, 2.1 km E Oxapampa	Hedges et. al (2008)	EU186695	–	–	–
2	*Pristimantis albertus*	RvM 527	Ingroup	Peru: Junin, Provincia Chanchamayo, Puyu Sacha	Lehr and von May (2017)	KY594751	–	–	–
3	*Pristimantis albertus*	MUSM 33299	Ingroup	Peru: Junin, Provincia Chanchamayo, Cerro San Pedro	Lehr and von May (2017)	KY594750	–	–	–
4	*Pristimantis albertus*	MUSM 33298	Ingroup	Peru: Junin, Provincia Chanchamayo, Cerro San Pedro	Lehr and von May (2017)	KY594749	–	–	–
5	*Pristimantis aniptopalmatus*	KU 261627	Ingroup	Peru: Pasco, 2.9 km N, 5.5 km E Oxapampa	Hedges et. al (2008)	EF493390	–	–	–
6	*Pristimantis aniptopalmatus*	KU 291666	Ingroup	Peru: Pasco, 2.9 km N, 5.9 km E Oxapampa	Hedges et. al (2008)	EU186694	–	–	–
7	*Pristimantis aniptopalmatus*	NMP6V 75053	Ingroup	Peru: Junin, Pui Pui	Lehr et. al (2017a)	KY006087	–	–	–
8	*Pristimantis aniptopalmatus*	NMP6V 75051	Ingroup	Peru: Junin, Pui Pui	Lehr et. al (2017a)	KY006086	–	–	–
9	*Pristimantis aniptopalmatus*	MUSM 31151	Ingroup	Peru: Pasco, Yanachaga-Chemillen	Lehr et. al (2017a)	KY006085	–	–	–
10	*Pristimantis aniptopalmatus*	MUSM 31130	Ingroup	Peru: Pasco, Yanachaga-Chemillen	Lehr et. al (2017a)	KY006084	–	–	–
11	*Pristimantis aniptopalmatus*	MUSM 31128	Ingroup	Peru: Pasco, Yanachaga-Chemillen	Lehr et. al (2017a)	KY006083	–	–	–
12	*Pristimantis aniptopalmatus*	MUSM 31111	Ingroup	Peru: Pasco, Yanachaga-Chemillen	Lehr et. al (2017a)	KY006082	–	–	–
13	*Pristimantis attenboroughi*	NMP6V 75529	Ingroup	Peru: Junin, near trail from Tasta to Tarhuish, Polylepis forest patch	Lehr and von May (2017)	KY594757	KY962784	KY962764	–
14	*Pristimantis attenboroughi*	NMP6V 75528	Ingroup	Peru: Junin, near trail from Tasta to Tarhuish, Polylepis forest patch	Lehr and von May (2017)	KY594756	KY962783	KY962763	–

N°	Species	Voucher	Group	Locality	Source	16 S	COI	RAG1	TYR
15	*Pristimantis attenboroughi*	NMP6V 75524	Ingroup	Peru: Junin, Upper part of Quebrada Tarhuish, 'Laguna Udrecocha'	Lehr and von May (2017)	KY594754	KY962781	KY962761	–
16	*Pristimantis attenboroughi*	NMP6V 75522	Ingroup	Peru: Junin, Quebrada Tarhuish, left bank of Antuyo River	Lehr and von May (2017)	KY594753	KY962780	KY962760	–
17	*Pristimantis attenboroughi*	MUSM 31186	Ingroup	Peru: Junin, Quebrada Tarhuish, left bank of Antuyo River	Lehr and von May (2017)	KY594752	KY962779	KY962759	–
18	*Pristimantis attenboroughi*	NMP6V 75525	Ingroup	Peru: Junin, Upper part of Quebrada Tarhuish, 'Laguna Udrecocha'	Lehr and von May (2017)	KY594755	KY962782	KY962762	–
19	*Pristimantis bounides*	NMP6V 75097	Ingroup	Peru: Junin, Quebrada Tasta, "Runda"	Lehr et. al (2017b)	KY962797	KY962790	KY962774	–
20	*Pristimantis bounides*	NMP6V 75066	Ingroup	"Peru: Junin, Sector Carrizal, Carrtera Satipo-Toldopampa, km 134"	Lehr et. al (2017b)	KY962796	–	KY962773	–
21	*Pristimantis bounides*	NMP6V 75540	Ingroup	"Peru: Junin, Sector Carrizal, Carrtera Satipo-Toldopampa, km 134"	Lehr et. al (2017b)	KY962795	–	KY962772	–
22	*Pristimantis bounides*	MUSM 31198	Ingroup	Peru: Junin, Quebrada Tasta, "Runda"	Lehr et. al (2017b)	KY962794	KY962789	KY962771	–
23	*Pristimantis cf aniptopalmatus*	VG-2017	Ingroup	Peru: Pasco, Yanachaga-Chemillen	Lehr et. al (2017a)	KY006088	–	–	–
24	*Pristimantis danae*	MNCN 44234	Ingroup	Peru: Cusco, Union, Valle de Kosnipata	Padial and De la Riva (2009)	EU192270	–	–	–
25	*Pristimantis danae*	IDLR 4001	Ingroup	Bolivia: La Paz: Santa Cruz de Valle Ameno	Padial and De la Riva (2009)	EU192260	–	–	–
26	*Pristimantis danae*	MNK-A 7182	Ingroup	Bolivia: La Paz, Huairuro, senda San Jose - Apolo	Padial and De la Riva (2009)	EU192261	–	–	–
27	*Pristimantis danae*	MNCN 43062	Ingroup	Bolivia: La Paz, Huairuro, senda San Jose - Apolo	Padial and De la Riva (2009)	EU192262	–	–	–
28	*Pristimantis danae*	MNCN 43069	Ingroup	Bolivia: La Paz: Arroyo Huacataya. senda San José y Apolo	Padial and De la Riva (2009)	EU192263	–	–	–
29	*Pristimantis danae*	MNK-A 7190	Ingroup	Bolivia: La Paz: Arroyo Huacataya. senda San José y Apolo	Padial and De la Riva (2009)	EU192264	–	–	–
30	*Pristimantis danae*	MNK-A 7273	Ingroup	Bolivia: La Paz: Serranía Bella Vista	Padial and De la Riva (2009)	EU192265	–	–	–
31	*Pristimantis danae*	IDLR 4815	Ingroup	Peru: Cusco: Unión, Valle de Kosñipata	Padial and De la Riva (2009)	EU192266	–	–	–
32	*Pristimantis danae*	MNCN 44232	Ingroup	Peru: Cusco: Unión, Valle de Kosñipata	Padial and De la Riva (2009)	EU192267	–	–	–
33	*Pristimantis danae*	MNCN 44233	Ingroup	Peru: Cusco: Unión, Valle de Kosñipata	Padial and De la Riva (2009)	EU192268	–	–	–
34	*Pristimantis danae*	IDLR 4822	Ingroup	Peru: Cusco: Unión, Valle de Kosñipata	Padial and De la Riva (2009)	EU192269	–	–	–
35	*Pristimantis danae*	IDLR 4824	Ingroup	Peru: Cusco: Unión, Valle de Kosñipata	Padial and De la Riva (2009)	EU192271	–	–	–
36	*Pristimantis danae*	IDLR 4825	Ingroup	Peru: Cusco: Unión, Valle de Kosñipata	Padial and De la Riva (2009)	EU192272	–	–	–
37	*Pristimantis danae*	MVZ 272358	Ingroup	Peru: Cusco: Valle de Kosñipata	von May et. al (2017)	KY652652	KY672984	KY672968	KY681073
38	*Pristimantis danae*	AC 141.09	Ingroup	Peru: Cusco: Valle de Kosñipata	**This paper**	**OR469891**	–	–	**OR542804**
39	*Pristimantis danae*	BL 37.13	Ingroup	Peru: Cusco: Valle de Kosñipata	**This paper**	**OR469893**	**OR478457**	**OR542831**	**OR542792**

N°	Species	Voucher	Group	Locality	Source	16 S	COI	RAG1	TYR
40	*Pristimantis danae*	BL 36.13	Ingroup	Peru: Cusco: Valle de Kosñipata	**This paper**	**OR469905**	**OR478456**	**OR542830**	**OR542791**
41	*Pristimantis humboldti*	NMP6V 75538	Ingroup	Peru: Junin, Quebrada Tarhuish, left bank of Antuyo River, Shiusha	Lehr et. al (2017b)	KY962799	KY962792	KY962776	–
42	*Pristimantis humboldti*	MUSM 31194	Ingroup	Peru: Junin, Quebrada Tarhuish, left bank of Antuyo River, Shiusha	Lehr et. al (2017b)	KY962798	KY962791	KY962775	–
43	*Pristimantis ornatus*	MTD 45073	Ingroup	Perú: Pasco: Oxapampa: Chinche: Cerca de Aquimarca	Hedges et. al (2008)	EU186660	–	–	–
44	*Pristimantis pharangobates*	KU 173492	Ingroup	Peru: Cusco: Buenos aires	Heinicke et. al (2007)	EF493706	–	–	–
45	*Pristimantis pharangobates*	MNCN 9494	Ingroup	Peru: Cusco: Valle de Kosñipata	Padial and De la Riva (2009)	FJ438802	–	–	–
46	*Pristimantis pharangobates*	MHNC 11451	Ingroup	Peru: Cusco: La Convención: Echarate: Urusayhua	**This paper**	**OR470757**	–	–	–
47	*Pristimantis pharangobates*	MHNC 11452	Ingroup	Peru: Cusco: La Convención: Echarate: Urusayhua	**This paper**	**OR471343**	–	–	–
48	*Pristimantis pharangobates*	AC 96.13	Ingroup	Peru: Cusco: Buenos Aires	**This paper**	**OR471423**	**OR478463**	–	**OR542798**
49	*Pristimantis pharangobates*	AC 9.13	Ingroup	Peru: Cusco: Buenos Aires	**This paper**	–	**OR478451**	**OR542824**	**OR542787**
50	*Pristimantis puipui*	NMP6V 75542	Ingroup	Peru: Junin, Pui Pui Protected Forest, Laguna Sinchon	von May and Lehr (2017b)	KY962800	–	KY962777	–
51	*Pristimantis reichlei*	MUSM 9267	Ingroup	Perú	Heinicke et. al (2007)	EF493707	–	EF493436	EF493498
52	*Pristimantis reichlei*	MNCN 43012	Ingroup	Bolivia: Cochabamba: Los Guácharos	Padial and De la Riva (2009)	EU192287	–	–	–
53	*Pristimantis reichlei*	MNK-A 6621	Ingroup	Bolivia: Cochabamba: Los Guácharos	Padial and De la Riva (2009)	EU192286	–	–	–
54	*Pristimantis reichlei*	MNCN 43249	Ingroup	Peru: Cusco: 5 km from San Lorenzo hacia Quince Mil	Padial and De la Riva (2009)	EU192288	–	–	–
55	*Pristimantis reichlei*	IDLR 4779	Ingroup	Peru: Puno: Entre Puerto Leguia y San Gabán	Padial and De la Riva (2009)	EU192285	–	–	–
56	*Pristimantis reichlei*	MUSM 26931	Ingroup	Peru: Huanuco: Panguana	Pinto-Sanchez et. al (2012)	JN991461	JN991392	–	–
57	*Pristimantis reichlei*	CORBIDI 16219	Ingroup	Peru: Cusco: Valle de Kosñipata	von May et. al (2017)	KY652657	KY672989	KY672972	KY681078
58	*Pristimantis reichlei*	AC 38.15	Ingroup	Peru: Cusco: Valle de Kosñipata	**This paper**	**OR471610**	**OR478458**	**OR542832**	–
59	*Pristimantis reichlei*	AC 113.12	Ingroup	Peru: Cusco: Valle de Kosñipata	**This paper**	**OR471611**	**OR478467**	**OR542839**	**OR542801**
60	*Pristimantis reichlei*	AC 20.15	Ingroup	Peru: Cusco: Valle de Kosñipata	**This paper**	**OR471619**	**OR478454**	**OR542828**	–
61	*Pristimantis reichlei*	AC 138.17	Ingroup	Peru: Quispicanchi: Soqtapata: Quincemil	**This paper**	**OR471620**	**OR478469**	**OR542841**	**OR542803**
62	*Pristimantis reichlei*	AC 75.17	Ingroup	Peru: Puno: PN Bahuaja-Sonene: Punto 4	**This paper**	**OR471646**	**OR478460**	**OR542833**	–
63	*Pristimantis reichlei*	AC 94.17	Ingroup	Peru: Puno: PN Bahuaja-Sonene: Punto 4	**This paper**	**OR471650**	**OR478462**	**OR542835**	–
64	*Pristimantis reichlei*	AC 16.17	Ingroup	Peru: Puno, Inambari, Santo Domingo	**This paper**	**OR471653**	–	**OR542826**	–
65	*Pristimantis reichlei*	AC 33.17	Ingroup	Peru: Puno, Inambari, Santo Domingo	**This paper**	**OR475320**	–	**OR542829**	–
66	*Pristimantis reichlei*	AC 147.17	Ingroup	Peru: Puno: carretera San Gaban-Ollachea: Casahuiri	**This paper**	**OR471655**	**OR478472**	**OR542844**	**OR542807**
67	*Pristimantis reichlei*	AC 10.14	Ingroup	Peru: Cusco: Pilcopata: Villa Carmen	**This paper**	**OR471656**	**OR478452**	**OR542825**	–
68	*Pristimantis reichlei*	AC 106.17	Ingroup	Peru: Puno: Isilluni: Valle Limbani	**This paper**	**OR472333**	**OR478465**	**OR542837**	**OR542799**

N°	Species	Voucher	Group	Locality	Source	16 S	COI	RAG1	TYR
69	*Pristimantis reichlei*	AC 109.17	Ingroup	Peru: Puno: Isilluni: Valle Limbani	**This paper**	**OR472388**	–	–	**OR542800**
70	*Pristimantis reichlei*	MNCN 4482	Ingroup	Peru: Cusco, Pantiacolla	Padial and De la Riva (2009)	EU712720	–	–	–
71	*Pristimantis reichlei*	NMP6V 72578	Ingroup	Bolivia: Pando, Bioceanica	Padial and De la Riva (2009)	EU712719	–	–	–
72	*Pristimantis rhabdolaemus*	FOCAM 34	Ingroup	Peru: Ayacucho: La Mar: Chiquintirca	**This paper**	**OR472495**	**OR478455**	**OR478455**	**OR542790**
73	*Pristimantis rhabdolaemus*	FOCAM 53	Ingroup	Peru: Ayacucho: La Mar: Toccate	**This paper**	**OR472500**	–	–	–
74	*Pristimantis rhabdolaemus*	FOCAM 76	Ingroup	Peru: Ayacucho: La Mar: Toccate	**This paper**	**OR472501**	**OR478461**	**OR542834**	**OR542797**
75	*Pristimantis rhabdolaemus*	FOCAM 145	Ingroup	Peru: Ayacucho: La Mar: Toccate	**This paper**	**OR472507**	–	–	–
76	*Pristimantis rhabdolaemus*	FOCAM 153	Ingroup	Peru: Ayacucho: La Mar: Toccate	**This paper**	**OR472508**	–	–	–
77	*Pristimantis rhabdolaemus*	FOCAM 361	Ingroup	Peru: Ayacucho: La Mar: Toccate	**This paper**	**OR472509**	–	**OR542849**	**OR542815**
78	*Pristimantis rhabdolaemus*	KNC 13	Ingroup	Peru: Ayacucho: Chungui: Chaupichaca	**This paper**	**OR472494**	–	–	**OR542788**
79	*Pristimantis rhabdolaemus*	KNC 47	Ingroup	Peru: Ayacucho: Chungui: Chaupichaca	**This paper**	**OR472498**	–	–	**OR542795**
80	*Pristimantis rhabdolaemus*	KNC 48	Ingroup	Peru: Ayacucho: Chungui: Chaupichaca	**This paper**	**OR472499**	–	–	**OR542796**
81	*Pristimantis rhabdolaemus*	KNC 103	Ingroup	Peru: Ayacucho: Chungui: Chaupichaca	**This paper**	**OR472502**	**OR478464**	**OR542836**	–
82	*Pristimantis rhabdolaemus*	KNC 106	Ingroup	Peru: Ayacucho: Chungui: Chaupichaca	**This paper**	**OR472503**	**OR478466**	**OR542838**	–
83	*Pristimantis rhabdolaemus*	KNC 118	Ingroup	Peru: Ayacucho: Chungui: Chaupichaca	**This paper**	**OR472505**	**OR478468**	**OR542840**	–
84	*Pristimantis rhabdolaemus*	KNC 119	Ingroup	Peru: Ayacucho: Chungui: Chaupichaca	**This paper**	**OR472506**	–	–	–
85	*Pristimantis rhabdolaemus*	KNC 116	Ingroup	Peru: Ayacucho: Chungui: Chaupichaca	**This paper**	**OR472504**	–	–	**OR542802**
86	*Pristimantis rhabdolaemus*	KNC 44	Ingroup	Peru: Ayacucho: Chungui: Chaupichaca	**This paper**	**OR472496**	–	–	**OR542793**
87	*Pristimantis rhabdolaemus*	KNC 45	Ingroup	Peru: Ayacucho: Chungui: Chaupichaca	**This paper**	**OR472497**	–	–	**OR542794**
88	*Pristimantis rhabdolaemus*	MHNC 17542	Ingroup	Peru: Cusco: La Convención: Echarate: Aendoshari	**This paper**	**OR472510**	**OR478479**	**OR542852**	–
89	*Pristimantis rhabdolaemus*	MUBI 17552	Ingroup	Peru: Cusco: La Convención: Echarate: Aendoshari	**This paper**	**OR472511**	–	–	**OR542819**
90	*Pristimantis sagittulus*	KU 261635	Ingroup	Peru: Pasco, 0.9 km N, 2.1 km E Oxapampa	Heinicke et. al (2007)	EF493705	–	EF493439	EF493501
91	*Pristimantis scitulus*	MUSM 41310	Ingroup	Peru: Ayacucho: Chungui: Chaupichaca	**This paper**	**OR469744**	–	**OR542823**	–
92	*Pristimantis scitulus*	MUSM 41309	Ingroup	Peru: Ayacucho: Chungui: Chaupichaca	**This paper**	**OR469328**	–	–	–
93	*Pristimantis scitulus*	MUSM 41312	Ingroup	Peru: Ayacucho: Chungui: Chaupichaca	**This paper**	**OR469801**	–	–	–
94	*Pristimantis scitulus*	MUSM 41313	Ingroup	Peru: Ayacucho: Chungui: Chaupichaca	**This paper**	**OR469804**	–	–	–
95	***Pristimantis similaris***	MUSM 41031	Ingroup	Peru: Ayacucho: La Mar: Cajadela	**This paper**	**OR478195**	**OR478473**	**OR542845**	**OR542808**
96	***Pristimantis similaris***	MUSM 41030	Ingroup	Peru: Ayacucho: La Mar: Cajadela	**This paper**	**OR478198**	**OR478475**	**OR542847**	**OR542812**
97	***Pristimantis similaris***	MUSM 41035	Ingroup	Peru: Ayacucho: La Mar: Cajadela	**This paper**	**OR478199**	**OR478476**	**OR542848**	**OR542813**
98	***Pristimantis similaris***	MUSM 41036	Ingroup	Peru: Ayacucho: La Mar: Cajadela	**This paper**	**OR478200**	–	**OR542851**	**OR542817**

N°	Species	Voucher	Group	Locality	Source	16 S	COI	RAG1	TYR
99	***Pristimantis similaris***	MUSM 41037	Ingroup	Peru: Ayacucho: Ayna: Machente	**This paper**	**OR478196**	–	–	**OR542810**
100	***Pristimantis similaris***	MUSM 41323	Ingroup	Peru: Ayacucho: Ayna: Machente	**This paper**	**OR478197**	–	–	**OR542811**
101	*Pristimantis sp.*	MVZ 272360	Ingroup	Peru: Cusco	von May et. al (2017)	KY652655	KY672987	KY681088	KY681076
102	*Pristimantis sp.*	MUSM 30433	Ingroup	Peru: Cusco	**This paper**	**OR478204**	–	**OR542855**	**OR542822**
103	*Pristimantis sp.*	MUSM 30418	Ingroup	Peru: Cusco	**This paper**	**OR478203**	–	**OR542854**	**OR542821**
104	*Pristimantis sp.*	AC 179.19	Ingroup	Peru: Cusco	**This paper**	–	–	**OR542846**	–
105	*Pristimantis sp.*	MUSM 27912	Ingroup	Peru: Cusco	**This paper**	**OR478202**	–	**OR542853**	**OR542820**
106	*Pristimantis sp.*	AC 365.07	Ingroup	Peru: Cusco	**This paper**	**OR478201**	**OR478478**	**OR542850**	**OR542816**
107	*Pristimantis sp3*	AMNH-A 165195	Ingroup	Bolivia: Santa Cruz: Caballero: Canton San José: Parque Nacional Amboro	Faivovich et. al (2005)	AY843586	–	–	–
108	*Pristimantis sp3*	MNK-A 6628	Ingroup	Bolivia: Santa Cruz: Serranía de la Siberia	Padial et. al (2007)	EU192258	–	–	–
109	*Pristimantis sp3*	MNCN 43036	Ingroup	Bolivia: Santa Cruz: La Yunga de Mairana	Padial et. al (2007)	EU192257	–	–	–
110	*Pristimantis stictogaster*	KU 291659	Ingroup	Peru: Pasco, 2.9 km N, 5.5 km E Oxapampa	Heinicke et. al (2007)	EF493704	–	EF493445	EF493506
111	*Pristimantis toftae*	KST 208	Ingroup	Peru: Huanuco, Puerto Inca, Panguana, Rio Yuyapichis (AKA Rio Llullapiches) near Rio Pachitea	Pinto-Sanchez et. al (2012)	JN991439	–	–	JN991566
112	*Pristimantis toftae*	KST 318	Ingroup	Peru: Huanuco, Puerto Inca, Panguana, Rio Yuyapichis (AKA Rio Llullapiches) near Rio Pachitea	**This paper**	**OR538542**	–	–	–
113	*Pristimantis toftae*	KU 215493	Ingroup	Peru: Madre de Dios: Cuzco Amazonico: 15 km E de Puerto Maldonado	Heinicke et. al (2007)	EF493353	–	–	–
114	*Pristimantis toftae*	MNCN 43025	Ingroup	Bolivia: Cochabamba: Los Guácharos	Padial and De la Riva (2009)	EU192293	–	–	–
115	*Pristimantis toftae*	MNCN 43246	Ingroup	Peru: Cusco: San Pedro, Valle de Marcapata	Padial and De la Riva (2009)	EU192294	–	–	–
116	*Pristimantis toftae*	AC 107.07	Ingroup	Peru: Cusco: Valle de Kosñipata	von May et. al (2017)	KY652659	KY672991	KY672974	KY681080
117	*Pristimantis toftae*	AC 19.15	Ingroup	Peru: Cusco: Valle de Kosñipata	**This paper**	**OR472575**	**OR478453**	**OR542827**	**OR542789**
118	*Pristimantis toftae*	CORBIDI 11889	Ingroup	Peru: Cusco: Valle de Kosñipata	**This paper**	**–**	–	–	**OR542818**
119	*Pristimantis toftae*	AC 144.16	Ingroup	Peru: Puno: Inambari: Santo Domingo	**This paper**	**OR472576**	**OR478470**	**OR542842**	**OR542805**
120	*Pristimantis toftae*	AC 147.16	Ingroup	Peru: Puno: Inambari: Santo Domingo	**This paper**	**OR472577**	**OR478471**	**OR542843**	**OR542806**
121	*Pristimantis bipunctatus*	MUSM 31120	Outgroup	Peru: Pasco, Yanachaga-Chemillen	Lehr et. al (2017a)	KY006089	–	–	–
122	*Pristimantis bipunctatus*	MUSM 31179	Outgroup	Peru: Junin, Pui Pui Protected Forest, Hito 3, Entrada del parque	Lehr and von May (2017)	KY594758	KY962785	KY962765	–
123	*Pristimantis bipunctatus*	KU 291638	Outgroup	Peru: Pasco, 0.7 km S, 4.5 km E Oxapampa	Heinicke et. al (2007)	EF493702	–	EF493430	EF493492
124	*Pristimantis iiap*	MUSM 40783	Outgroup	Peru: Ucayali: Pucallpa: Curimana	**This paper**	**OR470750**	**OR478474**	–	**OR542809**
125	*Pristimantis iiap*	MUSM 40841	Outgroup	Peru: Ucayali: Pucallpa: Curimana	**This paper**	**OR470749**	**OR478477**	–	**OR542814**
126	*Pristimantis prolatus*	KU 177433	Outgroup	Ecuador: Napo: Rio Salado	Hedges et. al (2008)	EU186701	–	–	–
127	*Pristimantis skydmainos*	MUSM 29286	Outgroup	Peru: Cusco: La Convención: Echarate	**This paper**	**OR469849**	–	–	–
128	*Pristimantis skydmainos*	448895	Outgroup	Peru	Heinicke et. al (2007)	EF493393	–	–	–

Appendix 3

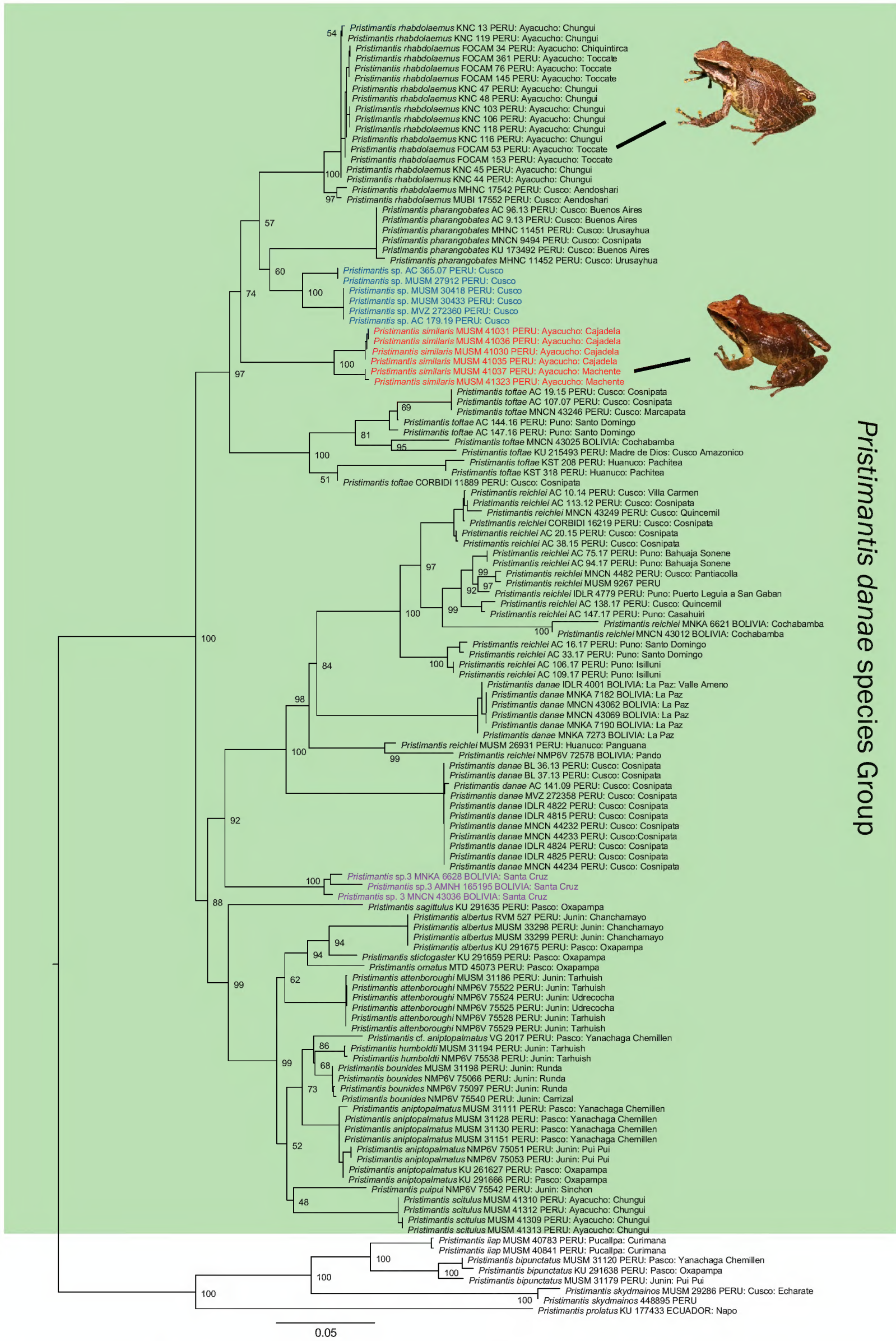
Pristimantis rhabdolaemus KNC 13 PERU: Ayacucho: Chungui
Pristimantis rhabdolaemus KNC 119 PERU: Ayacucho: Chungui
Pristimantis rhabdolaemus FOCAM 34 PERU: Ayacucho: Chiquintirca
Pristimantis rhabdolaemus FOCAM 361 PERU: Ayacucho: Toccate
Pristimantis rhabdolaemus FOCAM 76 PERU: Ayacucho: Toccate
Pristimantis rhabdolaemus FOCAM 145 PERU: Ayacucho: Toccate
Pristimantis rhabdolaemus KNC 47 PERU: Ayacucho: Chungui
Pristimantis rhabdolaemus KNC 48 PERU: Ayacucho: Chungui
Pristimantis rhabdolaemus KNC 103 PERU: Ayacucho: Chungui
Pristimantis rhabdolaemus KNC 106 PERU: Ayacucho: Chungui
Pristimantis rhabdolaemus KNC 118 PERU: Ayacucho: Chungui
Pristimantis rhabdolaemus KNC 116 PERU: Ayacucho: Chungui
Pristimantis rhabdolaemus FOCAM 53 PERU: Ayacucho: Toccate
Pristimantis rhabdolaemus FOCAM 153 PERU: Ayacucho: Toccate
Pristimantis rhabdolaemus KNC 45 PERU: Ayacucho: Chungui
Pristimantis rhabdolaemus KNC 44 PERU: Ayacucho: Chungui
Pristimantis rhabdolaemus MHNC 17542 PERU: Cusco: Aendoshari
Pristimantis rhabdolaemus MUBI 17552 PERU: Cusco: Aendoshari
Pristimantis pharangobates AC 96.13 PERU: Cusco: Buenos Aires
Pristimantis pharangobates AC 9.13 PERU: Cusco: Buenos Aires
Pristimantis pharangobates MHNC 11451 PERU: Cusco: Urusayhua
Pristimantis pharangobates MNCN 9494 PERU: Cusco: Cosnipata
Pristimantis pharangobates KU 173492 PERU: Cusco: Buenos Aires
Pristimantis pharangobates MHNC 11452 PERU: Cusco: Urusayhua
Pristimantis sp. AC 365.07 PERU: Cusco
Pristimantis sp. MUSM 27912 PERU: Cusco
Pristimantis sp. MUSM 30418 PERU: Cusco
Pristimantis sp. MUSM 30433 PERU: Cusco
Pristimantis sp. MVZ 272360 PERU: Cusco
Pristimantis sp. AC 179.19 PERU: Cusco
Pristimantis similaris MUSM 41031 PERU: Ayacucho: Cajadela
Pristimantis similaris MUSM 41036 PERU: Ayacucho: Cajadela
Pristimantis similaris MUSM 41030 PERU: Ayacucho: Cajadela
Pristimantis similaris MUSM 41035 PERU: Ayacucho: Cajadela
Pristimantis similaris MUSM 41037 PERU: Ayacucho: Machente
Pristimantis similaris MUSM 41323 PERU: Ayacucho: Machente
Pristimantis toftae AC 19.15 PERU: Cusco: Cosnipata
Pristimantis toftae AC 107.07 PERU: Cusco: Cosnipata
Pristimantis toftae MNCN 43246 PERU: Cusco: Marcapata
Pristimantis toftae AC 144.16 PERU: Puno: Santo Domingo
Pristimantis toftae AC 147.16 PERU: Puno: Santo Domingo
Pristimantis toftae MNCN 43025 BOLIVIA: Cochabamba
Pristimantis toftae KU 215493 PERU: Madre de Dios: Cusco Amazonico
Pristimantis toftae KST 208 PERU: Huanuco: Pachitea
Pristimantis toftae KST 318 PERU: Huanuco: Pachitea
Pristimantis toftae CORBIDI 11889 PERU: Cusco: Cosnipata
Pristimantis reichlei AC 10.14 PERU: Cusco: Villa Carmen
Pristimantis reichlei AC 113.12 PERU: Cusco: Cosnipata
Pristimantis reichlei MNCN 43249 PERU: Cusco: Quincemil
Pristimantis reichlei CORBIDI 16219 PERU: Cusco: Cosnipata
Pristimantis reichlei AC 20.15 PERU: Cusco: Cosnipata
Pristimantis reichlei AC 38.15 PERU: Cusco: Cosnipata
Pristimantis reichlei AC 75.17 PERU: Puno: Bahuaja Sonene
Pristimantis reichlei AC 94.17 PERU: Puno: Bahuaja Sonene
Pristimantis reichlei MNCN 4482 PERU: Cusco: Pantiacolla
Pristimantis reichlei MUSM 9267 PERU
Pristimantis reichlei IDLR 4779 PERU: Puno: Puerto Leguia a San Gaban
Pristimantis reichlei AC 138.17 PERU: Cusco: Quincemil
Pristimantis reichlei AC 147.17 PERU: Puno: Casahuiri
Pristimantis reichlei MNKA 6621 BOLIVIA: Cochabamba
Pristimantis reichlei MNCN 43012 BOLIVIA: Cochabamba
Pristimantis reichlei AC 16.17 PERU: Puno: Santo Domingo
Pristimantis reichlei AC 33.17 PERU: Puno: Santo Domingo
Pristimantis reichlei AC 106.17 PERU: Puno: Isilluni
Pristimantis reichlei AC 109.17 PERU: Puno: Isilluni
Pristimantis danae IDLR 4001 BOLIVIA: La Paz: Valle Ameno
Pristimantis danae MNKA 7182 BOLIVIA: La Paz
Pristimantis danae MNCN 43062 BOLIVIA: La Paz
Pristimantis danae MNCN 43069 BOLIVIA: La Paz
Pristimantis danae MNKA 7190 BOLIVIA: La Paz
Pristimantis danae MNKA 7273 BOLIVIA: La Paz
Pristimantis reichlei MUSM 26931 PERU: Huanuco: Panguana
Pristimantis reichlei NMP6V 72578 BOLIVIA: Pando
Pristimantis danae BL 36.13 PERU: Cusco: Cosnipata
Pristimantis danae BL 37.13 PERU: Cusco: Cosnipata
Pristimantis danae AC 141.09 PERU: Cusco: Cosnipata
Pristimantis danae MVZ 272358 PERU: Cusco: Cosnipata
Pristimantis danae IDLR 4822 PERU: Cusco: Cosnipata
Pristimantis danae IDLR 4815 PERU: Cusco: Cosnipata
Pristimantis danae MNCN 44232 PERU: Cusco: Cosnipata
Pristimantis danae MNCN 44233 PERU: Cusco:Cosnipata
Pristimantis danae IDLR 4824 PERU: Cusco: Cosnipata
Pristimantis danae IDLR 4825 PERU: Cusco: Cosnipata
Pristimantis danae MNCN 44234 PERU: Cusco: Cosnipata
Pristimantis sp.3 MNKA 6628 BOLIVIA: Santa Cruz
Pristimantis sp.3 AMNH 165195 BOLIVIA: Santa Cruz
Pristimantis sp. 3 MNCN 43036 BOLIVIA: Santa Cruz
Pristimantis sagittulus KU 291635 PERU: Pasco: Oxapampa
Pristimantis albertus RVM 527 PERU: Junin: Chanchamayo
Pristimantis albertus MUSM 33298 PERU: Junin: Chanchamayo
Pristimantis albertus MUSM 33299 PERU: Junin: Chanchamayo
Pristimantis albertus KU 291675 PERU: Pasco: Oxapampa
Pristimantis stictogaster KU 291659 PERU: Pasco: Oxapampa
Pristimantis ornatus MTD 45073 PERU: Pasco: Oxapampa
Pristimantis attenboroughi MUSM 31186 PERU: Junin: Tarhuish
Pristimantis attenboroughi NMP6V 75522 PERU: Junin: Tarhuish
Pristimantis attenboroughi NMP6V 75524 PERU: Junin: Udrecocha
Pristimantis attenboroughi NMP6V 75525 PERU: Junin: Udrecocha
Pristimantis attenboroughi NMP6V 75528 PERU: Junin: Tarhuish
Pristimantis attenboroughi NMP6V 75529 PERU: Junin: Tarhuish
Pristimantis cf. aniptopalmatus VG 2017 PERU: Pasco: Yanachaga Chemillen
Pristimantis humboldti MUSM 31194 PERU: Junin: Tarhuish
Pristimantis humboldti NMP6V 75538 PERU: Junin: Tarhuish
Pristimantis bounides MUSM 31198 PERU: Junin: Runda
Pristimantis bounides NMP6V 75066 PERU: Junin: Runda
Pristimantis bounides NMP6V 75097 PERU: Junin: Runda
Pristimantis bounides NMP6V 75540 PERU: Junin: Carrizal
Pristimantis aniptopalmatus MUSM 31111 PERU: Pasco: Yanachaga Chemillen
Pristimantis aniptopalmatus MUSM 31128 PERU: Pasco: Yanachaga Chemillen
Pristimantis aniptopalmatus MUSM 31130 PERU: Pasco: Yanachaga Chemillen
Pristimantis aniptopalmatus MUSM 31151 PERU: Pasco: Yanachaga Chemillen
Pristimantis aniptopalmatus NMP6V 75051 PERU: Junin: Pui Pui
Pristimantis aniptopalmatus NMP6V 75053 PERU: Junin: Pui Pui
Pristimantis aniptopalmatus KU 261627 PERU: Pasco: Oxapampa
Pristimantis aniptopalmatus KU 291666 PERU: Pasco: Oxapampa
Pristimantis puipui NMP6V 75542 PERU: Junin: Sinchon
Pristimantis scitulus MUSM 41310 PERU: Ayacucho: Chungui
Pristimantis scitulus MUSM 41312 PERU: Ayacucho: Chungui
Pristimantis scitulus MUSM 41309 PERU: Ayacucho: Chungui
Pristimantis scitulus MUSM 41313 PERU: Ayacucho: Chungui
Pristimantis iiap MUSM 40783 PERU: Pucallpa: Curimana
Pristimantis iiap MUSM 40841 PERU: Pucallpa: Curimana
Pristimantis bipunctatus MUSM 31120 PERU: Pasco: Yanachaga Chemillen
Pristimantis bipunctatus KU 291638 PERU: Pasco: Oxapampa
Pristimantis bipunctatus MUSM 31179 PERU: Junin: Pui Pui
Pristimantis skydmainos MUSM 29286 PERU: Cusco: Echarate
Pristimantis skydmainos 448895 PERU
Pristimantis prolatus KU 177433 ECUADOR: Napo
0.05
Pristimantis danae species Group

Figure A1. Maximum Likelihood tree non-collapsed of concatenated genes 16S rRNA, COI, RAG1 and TYR taken from GenBank and novel sequences. Numbers on nodes are bootstrap values (see Materials and Methods section for details). Green shadow corresponds to the ingroup. *Pristimantis similaris* sp. nov. in red, *Pristimantis* sp. 3 from Bolivia in purple and *Pristimantis* sp. from Cusco in blue.

Appendix 4

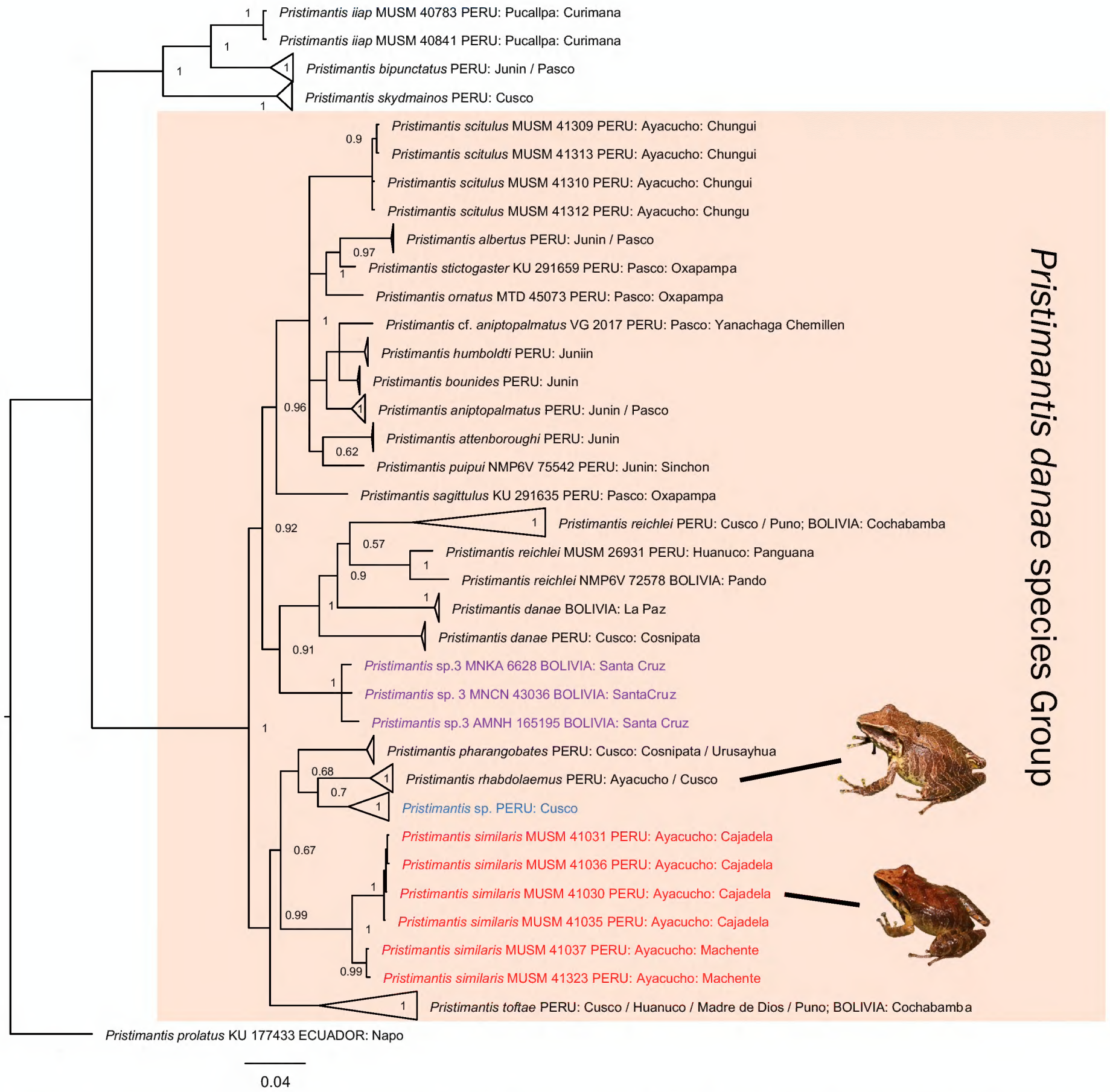
Pristimantis iiap MUSM 40783 PERU: Pucallpa: Curimana
Pristimantis iiap MUSM 40841 PERU: Pucallpa: Curimana
Pristimantis bipunctatus PERU: Junin / Pasco
Pristimantis skydmainos PERU: Cusco
Pristimantis scitulus MUSM 41309 PERU: Ayacucho: Chungui
Pristimantis scitulus MUSM 41313 PERU: Ayacucho: Chungui
Pristimantis scitulus MUSM 41310 PERU: Ayacucho: Chungui
Pristimantis scitulus MUSM 41312 PERU: Ayacucho: Chungu
Pristimantis albertus PERU: Junin / Pasco
Pristimantis stictogaster KU 291659 PERU: Pasco: Oxapampa
Pristimantis ornatus MTD 45073 PERU: Pasco: Oxapampa
Pristimantis cf. aniptopalmatus VG 2017 PERU: Pasco: Yanachaga Chemillen
Pristimantis humboldti PERU: Juniin
Pristimantis bounides PERU: Junin
Pristimantis aniptopalmatus PERU: Junin / Pasco
Pristimantis attenboroughi PERU: Junin
Pristimantis puipui NMP6V 75542 PERU: Junin: Sinchon
Pristimantis sagittulus KU 291635 PERU: Pasco: Oxapampa
Pristimantis reichlei PERU: Cusco / Puno; BOLIVIA: Cochabamba
Pristimantis reichlei MUSM 26931 PERU: Huanuco: Panguana
Pristimantis reichlei NMP6V 72578 BOLIVIA: Pando
Pristimantis danae BOLIVIA: La Paz
Pristimantis danae PERU: Cusco: Cosnipata
Pristimantis sp.3 MNKA 6628 BOLIVIA: Santa Cruz
Pristimantis sp. 3 MNCN 43036 BOLIVIA: SantaCruz
Pristimantis sp.3 AMNH 165195 BOLIVIA: Santa Cruz
Pristimantis pharangobates PERU: Cusco: Cosnipata / Urusayhua
Pristimantis rhabdolaemus PERU: Ayacucho / Cusco
Pristimantis sp. PERU: Cusco
Pristimantis similaris MUSM 41031 PERU: Ayacucho: Cajadela
Pristimantis similaris MUSM 41036 PERU: Ayacucho: Cajadela
Pristimantis similaris MUSM 41030 PERU: Ayacucho: Cajadela
Pristimantis similaris MUSM 41035 PERU: Ayacucho: Cajadela
Pristimantis similaris MUSM 41037 PERU: Ayacucho: Machente
Pristimantis similaris MUSM 41323 PERU: Ayacucho: Machente
Pristimantis toftae PERU: Cusco / Huanuco / Madre de Dios / Puno; BOLIVIA: Cochabamba
Pristimantis prolatus KU 177433 ECUADOR: Napo
0.04
Pristimantis danae species Group

Figure A2. Bayesian Tree phylogeny collapsed of concatenated genes 16S rRNA, COI, RAG1 and TYR. Numbers on nodes are posterior probabilities (see Materials and Methods section for details). Orange shadow corresponds to the ingroup. *Pristimantis similaris* sp. nov. in red, *Pristimantis* sp. 3 from Bolivia in purple and *Pristimantis* sp. from Cusco in blue.

Supplementary material 1

p-uncorrected distances of 591 pb including gaps of rRNA 16s gene

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Data type: xls

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